Drug Discovery

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Structure-Based Discovery of Natural-Product-like TNF-α Inhibitors**

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Tumor necrosis factor α (TNF-α) is a multifunctional cytokine that acts as a central biological mediator for critical immune functions, including inflammation, infection, and antitumor responses.[1] Dysregulation of TNF-α has been implicated in cases of tumorigenesis, diabetes, and especially in autoinflammatory diseases such as rheumatoid arthritis, psoriatic arthritis, and Crohn's disease.[2] The synthetic antibodies etanercept, infliximab, and adalimumab, approved for the treatment of inflammatory diseases bind to TNF-α directly, preventing its association with the tumor necrosis factor receptor (TNFR).[3] However, their potential to cause serious side effects such as eliciting an autoimmune anti-antibody response or the weakening of the body's immune defenses to opportunistic infections, has stimulated the development of alternative small-molecule-based therapies to TNF-α inhibition.[4] Most such small-molecule inhibitors reported in the literature target TNF-α indirectly.[5–8]

To our knowledge, the only small molecules capable of antagonizing TNF-α directly are the polysulfonated naphthylurea suramin and its analogues,[9] and the indole-linked chromone designated SPD304 (Figure 1).[10] Unfortunately, the low potency and poor selectivity of suramin coupled with its tendency to cause adverse side effects renders it unsuitable for anti-TNF-α therapies.[11] Furthermore, SPD304, containing the toxic 3-alkylindole moiety, was found to be metabolized by cytochrome P450 enzymes through a dehydrogenation pathway similar to that of the potent pneumotoxin 3-methylindole, producing reactive electrophilic iminium species capable of reacting with protein and DNA targets.[12] Therefore, the development of relatively less toxic small-molecule inhibitors of TNF-α for therapeutic applications remains a highly desirable goal.

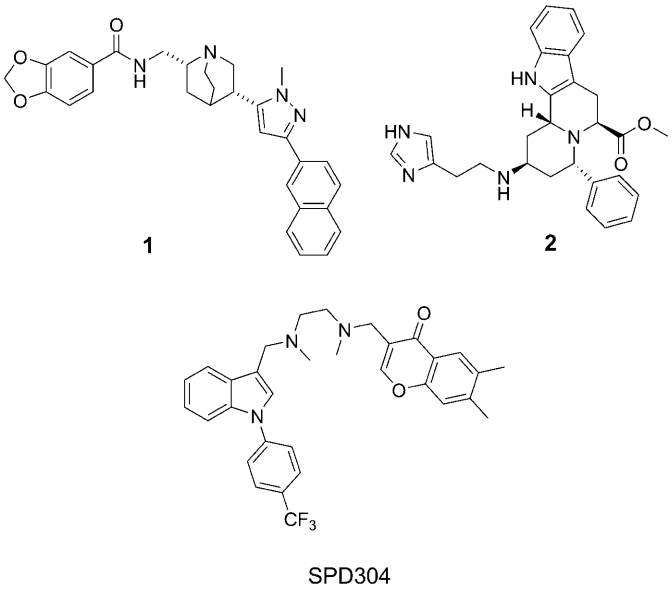

Figure 1. Chemical structures of small-molecule TNF-α inhibitors quinuclidine **1**, indoloquinolizidine **2**, and SPD304.

Natural products (NPs) have been refined over evolutionary time scales for optimal interactions with biomolecules. Not surprisingly, NPs have represented a cornerstone of pharmaceutical research, as they offer a diverse range of chemical scaffolds, bioactive substructures, and potentially lower toxicity profiles.[13] Historically, many approved drugs have been NPs, whereas numerous others were derived from or inspired by a NP template.[14] Encouraged by these ideas, and by the relative dearth of potent and nontoxic small-molecule inhibitors directly targeting TNF-α, we sought to apply high-throughput, ligand-docking-based virtual screening methods to identify TNF-α inhibitors from natural-product chemical libraries. We used the X-ray cocrystal structure of a TNF-α dimer with SPD304 (PDB code: 2AZ5)[10] as the molecular model for our investigation.

Like most protein–protein interfaces, the binding pocket of the TNF-α dimer is relatively large and featureless, and lacks clearly defined binding crevices or mechanism-based contacts.[15] The binding site is mostly hydrophobic, consisting primarily of glycine, leucine, and tyrosine residues. Not unexpectedly, the binding interaction of the small molecule SPD304 to TNF-α has been described to be predominantly hydrophobic and shape-driven.[10] Small-molecule inhibitors of TNF-α should be therefore relatively hydrophobic and large enough to interact with both subunits of the TNF-α dimer simultaneously to prevent the binding of the third subunit, which forms the biologically active trimer complex.

Over 20000 compounds from a chemical library of natural-product and natural-product-like structures[16] were screened in silico. The continuously flexible ligands were docked to a grid representation of the receptor and assigned a score reflecting the quality of the complex according to the internal coordinate mechanics (ICM) method [ICM-Pro 3.6-

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1d molecular docking software (Molsoft)].[18] The highest-scoring 16 compounds from the virtual screening results were tested in a preliminary ELISA to assess their ability to inhibit the binding of TNF-α to TNFR-1. Two chemically distinct structures, the pyrazole-linked quinuclidine **1** and the indolo-[2,3-*a*]quinolizidine **2**, emerged as the top candidates (Figure 1). The binding poses of these two compounds overlap well with the crystallographic pose of SPD304 to TNF-α (Figure 2). Like SPD304, compounds **1** and **2** are large enough to interact with the residues from both subunits of the TNF-α dimer, thereby occupying and blocking the binding site for the third TNF-α subunit.

In the top-scoring binding mode of **1** to the TNF-α dimer, the pyrazole-linked quinuclidine substructure occupies the hydrophobic binding pocket, and the dioxolane oxygen atom of **1** forms a hydrogen bond with the backbone amino group of Gly121 of TNF-α subunit B (Figure 2a). Compound **2** is not predicted to occupy the region of space close to Gly121 of subunit B, but instead forms a hydrogen bond with the side-chain hydroxy group of Tyr151 of subunit B through its imidazole functionality (Figure 2b). Common features of the predicted binding modes of **1**, **2**, and SPD304 are the extended hydrophobic ring systems that are in contact with the β strand (Leu120-Gly121-Gly122) of TNF-α subunit A, and the presence of polar functional groups oriented away from the binding pocket and exposed to the aqueous environment. Interestingly, whereas the indole substructures of **2** and SPD304 (Figure 2c) are located in a similar region of space, their orientations with respect to the β strand of subunit A are different. The lack of salt bridges or hydrogen-bonding networks in our models of **1** and **2** with TNF-α is consistent with previous findings that the interaction between the small molecule SPD304 and TNF-α is primarily hydrophobic and shape-driven.[10] The calculated binding scores of −34.7 and −36.4 for **1** and **2**, respectively, reflect a strong interaction between the compounds and the dimer complex. As a reference, we calculated the binding score of SPD304 to be −32.9. The predicted binding coordinates of SPD304 in the binding pocket are within 1.0 Å root-mean-square deviation of the reported values derived from the protein X-ray crystal structure.[10]

The quinuclidine core of **1** is present in a variety of natural products, such as the antimalarial cinchona alkaloids.[18] Natural products containing the indolo[2,3-*a*]quinolizidine scaffold of **2** include the alkaloids geissoschizine, deplancheine, corynantheidine, and yohimbane.[19] Waldmann and co-workers employed a biology-oriented synthetic approach to generate indolo[2,3-*a*]quinolizidine inhibitors of mycobacterial protein tyrosine phosphatase B.[20] To the best of our knowledge, no TNF-α-binding activity nor any other biological activity of **1** or **2** has been reported in the literature.

To validate the results of our molecular modeling, we performed dose-response experiments with compounds **1** and **2** to determine their half-maximal inhibitory concentration (IC_{50}) values against the TNF-α–TNFR-1 interaction using an ELISA (Figure 3). Encouragingly, indoloquinolizidine **2** (IC_{50} ≈ 10 μM) was found to be more active than SPD304, the most potent small-molecule TNF-α inhibitor reported to date (IC_{50} = 22 μM by a comparable ELISA).[10,21] Quinuclidine **1** was moderately active against TNF-α with an IC_{50} value of approximately 50 μM.

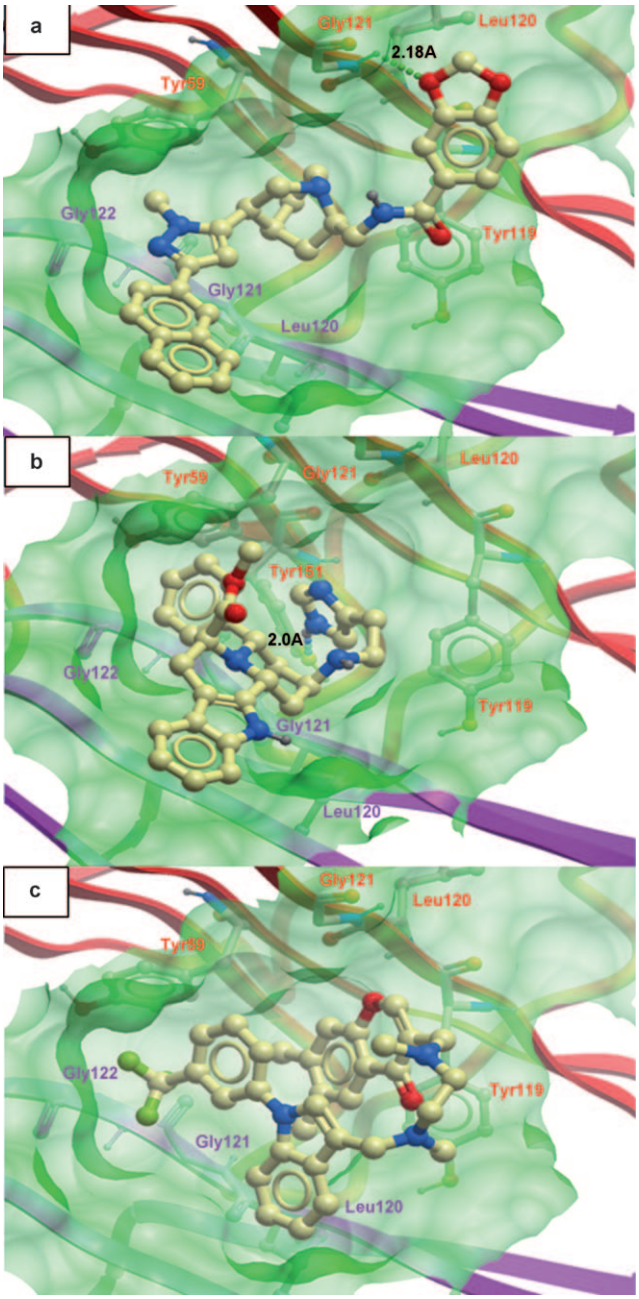

Figure 2. Low-energy binding conformations of a) **1**, b) **2**, and c) SPD304 bound to the TNF-α dimer generated by virtual ligand docking. The two subunits of the TNF-α dimer are depicted in ribbon form and are colored purple (subunit A) and red (subunit B). The small molecules are depicted as ball-and-stick models showing carbon (yellow), hydrogen (grey), oxygen (red), nitrogen (blue), and fluoride (green) atoms. Hydrogen bonds are depicted as dotted lines. The binding pocket of the TNF-α dimer is represented as a translucent green surface.

We next investigated the ability of compounds **1** and **2** to inhibit TNF-α signaling in human cells. TNF-α solutions pre-incubated with the test compound were added to HepG2 cells, which were stably transfected with the NF-κB-luciferase gene.

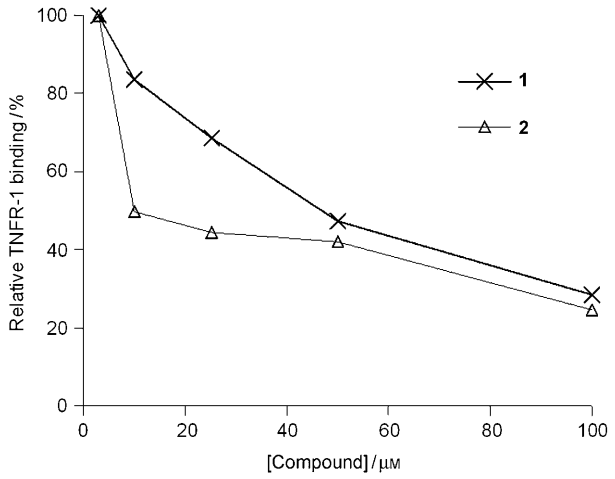

Figure 3. Compound inhibition of TNFR-1 binding to immobilized TNF-α (ELISA). Microtiter plates coated with TNF-α were incubated with TNFR-1 together with **1** or **2** at the indicated concentrations. TNFR-1 binding was detected using an anti-TNFR antibody and horseradish peroxidase conjugated secondary antibody. Approximate IC_{50} values: **1**: 10 μM and **2**: 50 μM.

The inhibition of TNF-α-induced NF-κB signaling by the test compound was detected by monitoring the reduction in the luciferase activity of the cell lysates (Figure 4). Surprisingly, indoloquinolizidine **2** ($IC_{50} > 30$ μM) was found to be less active than quinuclidine **1** ($IC_{50} \approx 5$ μM) in the cellular luciferase assay, despite showing greater potency in the cell-free ELISA. Notably, **2** exhibited a IC_{50} value similar to that of SPD304 ($IC_{50} \approx 3$ μM) as measured by our system, although it was less potent than SPD304 at higher concentrations. We hypothesize that the markedly reduced activity of **2** in the cell culture could be because of its low bioavailability resulting from either poor cellular uptake or metabolic degradation of **2**.[22]

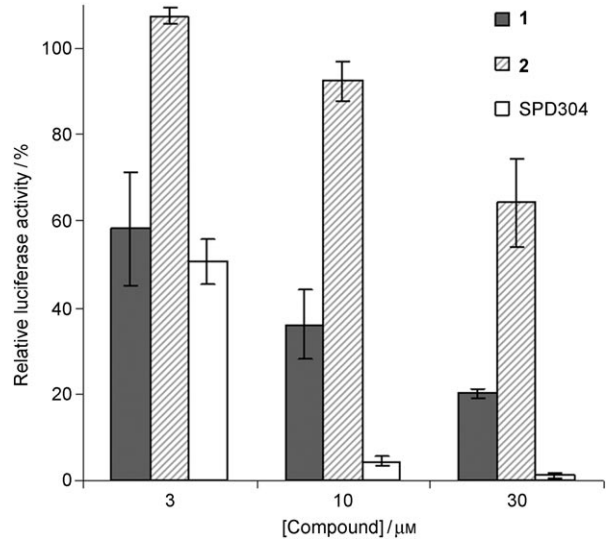

Figure 4. Compound inhibition of cellular TNF-α-induced NF-κB activity. HepG2 cells stably transfected with the NF-κB-luciferase gene were stimulated with TNF-α pre-incubated with the indicated concentrations of **1**, **2**, or SPD304. Cell lysates were analyzed for luciferase activity to determine the extent of NF-κB inhibition. Approximate IC_{50} values: **1**: 5 μM, **2**: > 30 μM, and SPD304: 3 μM.

In conclusion, we have discovered two small-molecule TNF-α inhibitors from a natural-product and natural-product-like chemical libraries using structure-based design. The identification of quinuclidine **1** and quinolizine **2** represents, to the best of our knowledge, only the third and fourth examples of the direct targeting of TNF-α by a small molecule. Importantly, indoloquinolizidine **2** ($IC_{50} \approx 10$ μM) was found to be more potent against TNF-α in the ELISA compared to SPD304, the strongest small-molecule TNF-α inhibitor reported to date. Quinuclidine **1** ($IC_{50} \approx 5$ μM) displayed activity comparable to that of SPD304 ($IC_{50} \approx 3$ μM) against cellular TNF-α induced NF-κB signaling. We are currently conducting computer-based hit-to-lead optimization to generate additional analogues for in vitro testing.

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[1] H. Wajant, K. Pfizenmaier, P. Scheurich, *Cell Death Differ.* **2003**, *10*, 45–65.
[2] B. B. Aggarwal, *Nat. Rev. Immunol.* **2003**, *3*, 745–756.
[3] K. Chatzantoni, A. Mouzaki, *Curr. Top. Med. Chem.* **2006**, *6*, 1707–1714.
[4] M. A. Palladino, F. R. Bahjat, E. A. Theodorakis, L. L. Moldawer, *Nat. Rev. Drug Discovery* **2003**, *2*, 736–746.
[5] S. Haraguchi, N. K. Day, W. Kamchaisatian, M. Beigier-Pompadre, S. Stenger, N. Tangsinmankong, J. W. Sleasman, S. V. Pizzo, G. J. Cianciolo, *AIDS Res. Ther.* **2006**, *3*, 8.
[6] H. Rasmussen, P. P. McCann, *Pharmacol. Ther.* **1997**, *75*, 69–75.
[7] M. R. Lee, C. Dominguez, *Curr. Med. Chem.* **2005**, *12*, 2979–2994.
[8] a) J. R. Burke, M. A. Pattoli, K. R. Gregor, P. J. Brassil, J. F. MacMaster, K. W. McIntyre, X. Yang, V. S. Iotzova, W. Clarke, J. Strnad, Y. Qiu, F. Zusi, *J. Biol. Chem.* **2003**, *278*, 1450–1456; b) C. H. Leung, S. P. Grill, W. Lam, W. Gao, H. D. Sun, Y. C. Cheng, *Mol. Pharmacol.* **2006**, *70*, 1946–1955.
[9] a) R. Alzani, A. Cortin, L. Grazioli, E. Cozzi, P. Ghezzi, F. Marcucci, *J. Biol. Chem.* **1993**, *268*, 12526–12529; b) F. Mancini, C. M. Toro, M. Mabilia, M. Giannangeli, M. Pinza, C. Milanese, *Biochem. Pharmacol.* **1999**, *58*, 851–859.
[10] M. M. He, A. S. Smith, J. D. Oslob, W. M. Flanagan, A. C. Braisted, A. Whitty, M. T. Cancilla, J. Wang, A. A. Lugovskoy, J. C. Yoburn, A. D. Fung, G. Farrington, J. K. Eldredge, E. S. Day, L. A. Cruz, T. G. Cachero, S. K. Miller, J. E. Friedman, I. C. Choong, B. C. Cunningham, *Science* **2005**, *310*, 1022–1025.
[11] R. P. McGeary, A. J. Bennett, Q. B. Tran, K. L. Cosgrove, B. P. Ross, *Mini. Rev. Med. Chem.* **2008**, *8*, 1384–1394.
[12] H. Sun, G. S. Yost, *Chem. Res. Toxicol.* **2008**, *21*, 374–385.
[13] a) P. Ertl, S. Roggo, A. Schuffenhauer, *J. Chem. Inf. Model.* **2008**, *48*, 68–74; b) R. Breinbauer, I. R. Vetter, H. Waldmann, *Angew. Chem.* **2002**, *114*, 3002–3015; *Angew. Chem. Int. Ed.* **2002**, *41*, 2878–2890.
[14] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461–477.
[15] P. L. Toogood, *J. Med. Chem.* **2002**, *45*, 1543–1558.

[16] Obtained from AnalytiCon Discovery. See the Supporting Information for details.
[17] M. Totrov, R. Abagyan, *Proteins Suppl.* **1997**, *29*, 215 – 220.
[18] T. S. Kaufman, E. A. Rúveda, *Angew. Chem.* **2005**, *117*, 876 – 907; *Angew. Chem. Int. Ed.* **2005**, *44*, 854 – 885.
[19] J. E. Saxton, *Nat. Prod. Rep.* **1997**, *14*, 559 – 590.
[20] I. R. Corrêa, Jr., A. Nören-Müller, H.-D. Ambrosi, S. Jakupovic, K. Saxena, H. Schwalbe, M. Kaiser, H. Waldmann, *Chem. Asian J.* **2007**, *2*, 1109 – 1126.
[21] In a side-by-side experiment, we measured the inhibition of TNFR-1 binding to SPD304 (10 μM) to be 34%.
[22] Using Molsoft's molecular property prediction tool, we calculated **2** to be less "drug-like" than **1**. See the Supporting Information for details.

