STRUCTURE-BASED DISCOVERY OF NOVEL NIK INHIBITORS

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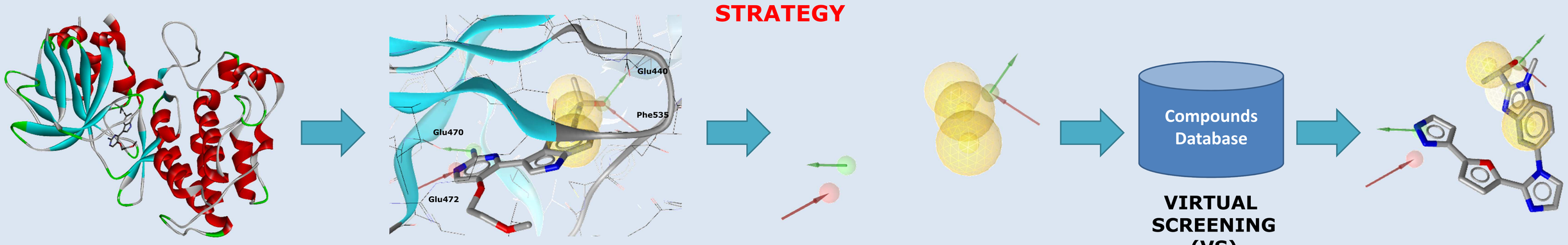
INTRODUCTION

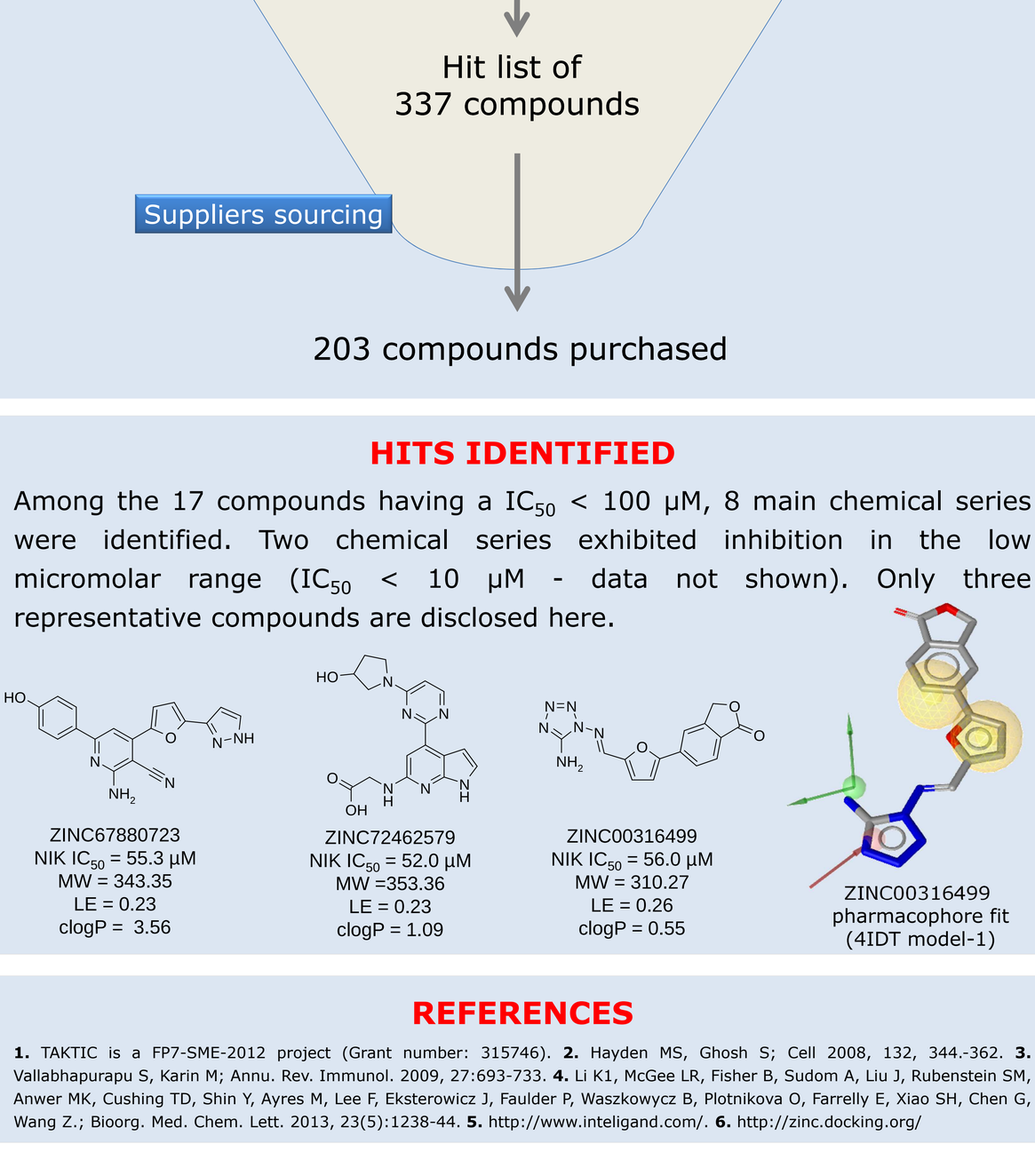
The primary objective of the TAKTIC (TrAnslational Kinase Tumor Inhibitor discovery Consortium) project is to identify and develop highly specific small molecule inhibitors targeting the kinases IKKα, IKKβ and NIK.[1] NF-κB-inducing kinase (NIK) is a serine/threonine protein kinase essential for the activation of a second major NF-κB (NFκB2) pathway which controls several critical biological functions such as inflammation or cell survival.[2-3] The X-ray structure of NIK, a mitogen-activated protein kinase kinase kinase (MAP3K), has recently been published and was used in this work for a structure-based ligand design approach.[4] Using this strategy, we identified a series of new small molecule inhibitors of NIK.

STRATEGY



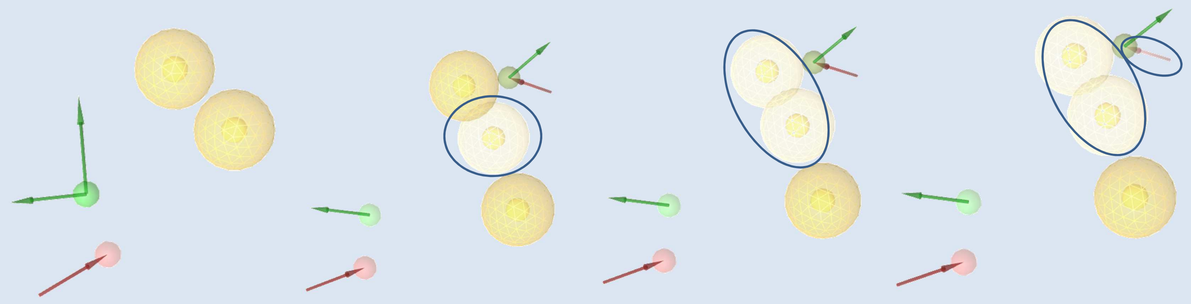
PDB ID: 4IDT, 4IDV | **Protein-ligand interactions** | **Pharmacophore model** | **Virtual hits**

HIT DISCOVERY FLOWCHART



STRUCTURE-BASED PHARMACOPHORE MODELS AND VS

Two xray structures (PDB entries 4IDT and 4IDV) were analyzed and used to generate ten pharmacophore models by using the LigandScout software.[5] Models were tuned and optimized in order to obtain an optimal hit rate for the virtual screening. After validation by using a set of active ligands,[4] five models were selected for virtual screening of two 3D molecular structure databases (Zinc databases: drug-now and frag-now).[6]

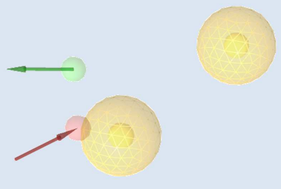


4IDT model-1

4IDV model-2
Hydrophobe feature disabled

4IDV model-5
2 Hydrophobe features disabled

4IDV model-7
2 Hydrophobe + 1 HBA features disabled



cpd14-4IDV model-2
1 HBA + 1 HBD features added

Models	Zinc database 6.3 Mi cpds	Zinc fragments 700 K cpds
4IDT model-1	20 397	2 122
4IDV model-2	95	3
4IDV model-5	276	24
4IDV model-7	1459	64
cpd14-4IDV model-2	54	5

20 779 virtual hits

BIOCHEMICAL ASSAYS

Human full length NIK kinase was expressed in Sf9 insect cells and purified as recombinant GST fusion protein. NIK kinase activity was measured using the radiometric 33PanQinase® activity assay (ProQinase GmbH). In a primary screen, 203 compounds were tested for NIK inhibition (% residual activity) at 100 µM. Verification of 27 primary hit compounds showing at least 40% NIK inhibition at 100 µM was done by determination of IC_{50} values (concentration range: 3nM - 100 µM). 17 compounds showed a IC_{50} below 100 µM and several highly active inhibitors were identified (IC_{50} < 10 µM).

HITS IDENTIFIED

Among the 17 compounds having a IC_{50} < 100 µM, 8 main chemical series were identified. Two chemical series exhibited inhibition in the low micromolar range (IC_{50} < 10 µM - data not shown). Only three representative compounds are disclosed here.

ZINC67880723
NIK IC_{50} = 55.3 µM
MW = 343.35
LE = 0.23
clogP = 3.56

ZINC72462579
NIK IC_{50} = 52.0 µM
MW =353.36
LE = 0.23
clogP = 1.09

ZINC00316499
NIK IC_{50} = 56.0 µM
MW = 310.27
LE = 0.26
clogP = 0.55

ZINC00316499
pharmacophore fit
(4IDT model-1)

CONCLUSION

Pharmacophore-based virtual screening experiments were performed with a large chemical database allowing, after selection and experimental evaluation of 203 compounds, to identify micromolar hits against NIK kinase. Two chemical series (IC_{50} < 10 µM) are currently under investigation in a hit-to-lead validation process, kinase selectivity (profiling) and early ADMET evaluation. The objective is to enter into a lead optimization phase in order to develop new and selective NIK small molecule inhibitors.

REFERENCES

1. TAKTIC is a FP7-SME-2012 project (Grant number: 315746). **2.** Hayden MS, Ghosh S; Cell 2008, 132, 344.-362. **3.** Vallabhapurapu S, Karin M; Annu. Rev. Immunol. 2009, 27:693-733. **4.** Li K1, McGee LR, Fisher B, Sudom A, Liu J, Rubenstein SM, Anwer MK, Cushing TD, Shin Y, Ayres M, Lee F, Eksterowicz J, Faulder P, Waszkowycz B, Plotnikova O, Farrelly E, Xiao SH, Chen G, Wang Z.; Bioorg. Med. Chem. Lett. 2013, 23(5):1238-44. **5.** http://www.inteligand.com/. **6.** http://zinc.docking.org/