Anti-inflammatory Library

8,203 Compounds

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Preamble:

The accumulated scientific knowledge has already revealed key biological targets, such as COX2, and related pro-inflammatory mediators (cytokines and chemokines, ILs, TNF-α, MIF, IFN-γ, MMPs) □ implicated in uncontrolled, destructive inflammatory reaction. A number of physiologically active agents are currently approved for market or under active investigation in different clinical trials. However, recent findings have exposed the fatal side effects directly associated with drug-therapy based on COX-2 inhibition. Considering these possible harmful outcomes, a range of novel therapeutically relevant biological targets which particularly includes NF-κB and JAK/STAT signaling pathways has received a growing attention. Here we present our original anti-inflammatory (AI) focused library which includes small molecule compounds targeted specifically against the biological targets just mentioned.

Introduction:

In the original conception, inflammation (Latin, *inflammatio*, to set on fire) is an extremely complex biological response of the human organism to a variety of harmful stimuli, such as pathogens, foreign substances (bacteria and viruses), damaged cells and wounds, infection, viruses and irritants; the body sends out signals that normal tissues are infected, damaged or somehow abnormal. However, in some diseases, the immune system inappropriately triggers an inflammatory response with no foreign substances or infection presence. In these autoimmune conditions the body's defense mechanism causes huge damage to its own tissues sustaining the perpetual or transitory activation of the inflammatory process. Abnormalities associated with uncontrolled inflammation comprise a large, unrelated group of disorders which underlie a variety of dangerous human diseases, such as rheumatoid arthritis (RA), ischaemic heart disease, atherosclerosis, cancer, fibrosis, hay fever as well as several neurodegenerative disorders including Parkinson's and Alzheimer's diseases. To avoid these harmful outcomes, inflammation is normally tightly controlled by a variety of regulating factors.

Among a huge number of pro-inflammatory mediators cyclo-oxygenase 1 and 2 (COX-1/-2), mitogen-activated protein kinases (MAPKs), janus protein tyrosine kinases (JAKs), nuclear transcription factor (NF-κB) and signal transducers and activators of transcription (STAT) are the most principal effectors that directly or indirectly lead to the production of a vast number of pro-inflammatory cytokines and regulatory proteins such as IL-1/6, TNF-α, MIF, IFN-γ, MMPs.[i] These messengers, in turn, jointly support inflammatory processes providing both homeostatic as well as pathological outcomes.

Considering a wide spectrum of potential risks directly related with `classical` anti-inflammatory therapy that is commonly based on the use of non-steroidal anti-inflammatory drugs (NSAIDs) and COX1/2 inhibitors the advanced generation of novel anti-inflammatory drugs primarily acting against pro-inflammatory mediator production are being discovered and developed based on their effects on alternative signal transduction pathways. These drugs are currently being heralded as the new-age therapies to control those diseases where these factors and signaling molecules as well as other nonprostaglandin components of chronic inflammatory and neurodegenerative diseases are becoming manifest. What started out as drugs to control inflammation, pain and fever in the last two centuries now has exploded to reveal an enormous range and type of anti-inflammatory agents and discovery of new prominent therapeutic targets to treat a whole range of conditions that were never hitherto envisaged. Among these biological targets NF-κB and JAK/STAT signaling molecules represent the most promising avenue for achieving optimal therapeutic response with minimal side effects.

1. NF-κB Signalling Pathway

The NF-κB intracellular signaling system seems to be becoming the dominant paradigm for specific signal transduction molecules, regulatory proteins and gene activation in response to inflammatory and menacing stimuli. Especially during initial hyper-inflammatory states of an acute illness such as sepsis or in the course of chronic inflammation and autoimmune diseases inhibition of IKK-driven NF-κB activation provides a promising therapeutic strategy. The spectrum of NF-κB target genes include primarily those that are responsible for mediators and effectors of both innate and adaptive immunity and inhibitors of apoptosis, growth promoting factors and virus-encoded proteins involved in viral replication, as well as self-regulatory proteins for NF-κB actions.[ii] In addition to the original inflammatory conditions, NF-κB signaling pathway deeply involves in the onset of various inflammatory-related autoimmune disorders and different types of cancer.[iii] Thus, it has recently been

reported that NF-κB plays major roles in leukemia, inflammatory bowel disease, arthritis, sepsis, asthma, multiple sclerosis, colitis, diabetic neuropathy[iv] and AIDS. For example, RA pathology was found to be thoroughly mediated by a number of cytokines (TNF-α, IL-1/6/17, IFN-γ, etc.), chemokines (MCP-1/4, CCL18, etc.), cell adhesion molecules (ICAM-1, VCAM-1, etc.) and MMPs. Thus, in patients diagnosed with RA, activation of NF-κB signaling pathway results in the transcription of a multitude of responsive genes that contribute to the inflammatory phenotype, including TNF-α, IL-6 and MMPs that, in turn, recruit immune cells to the inflamed pannus. This is largely a consequence of activation of the canonical NF-κB pathway that leads finally to the formation of heterodimeric transcriptional units composed of different p/p complexes which directly initiate gene expression.[v] A schematic diagram of the canonical and alternative NF-κB pathways is shown in Fig. 1.

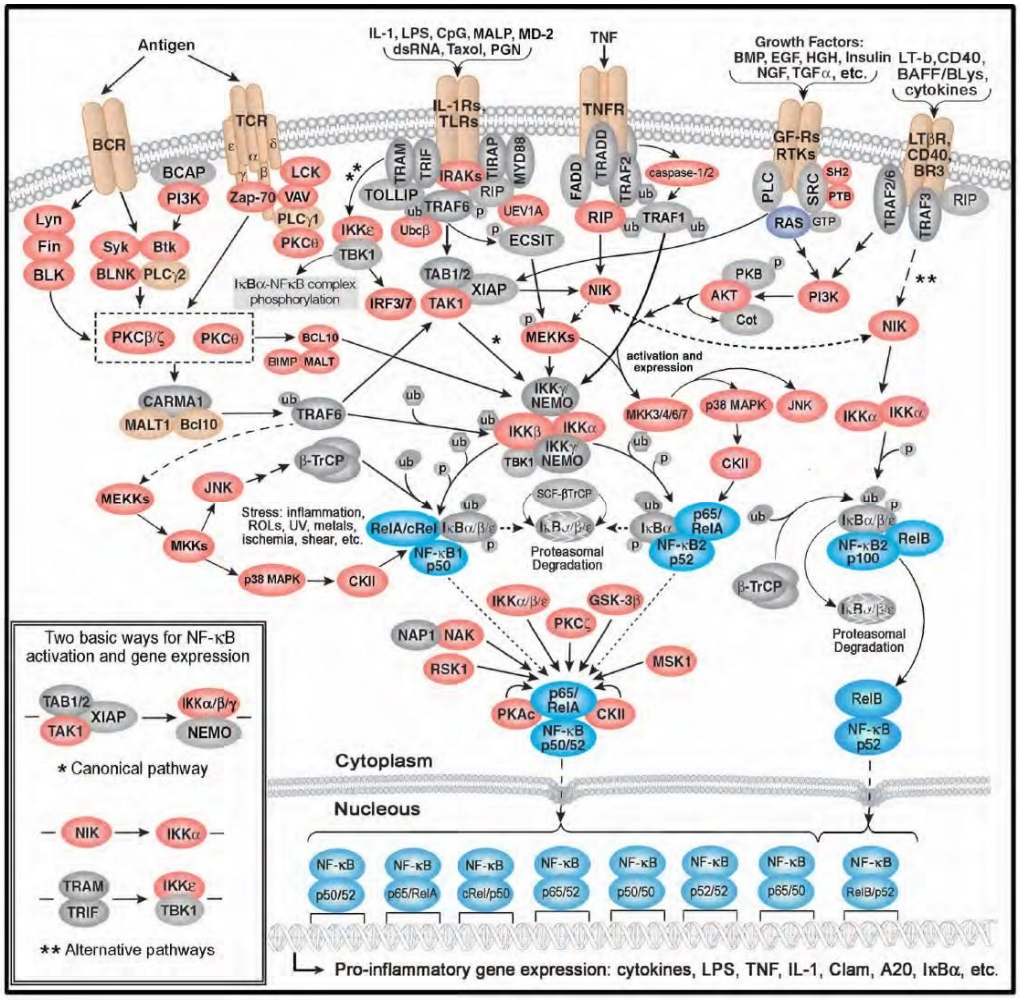
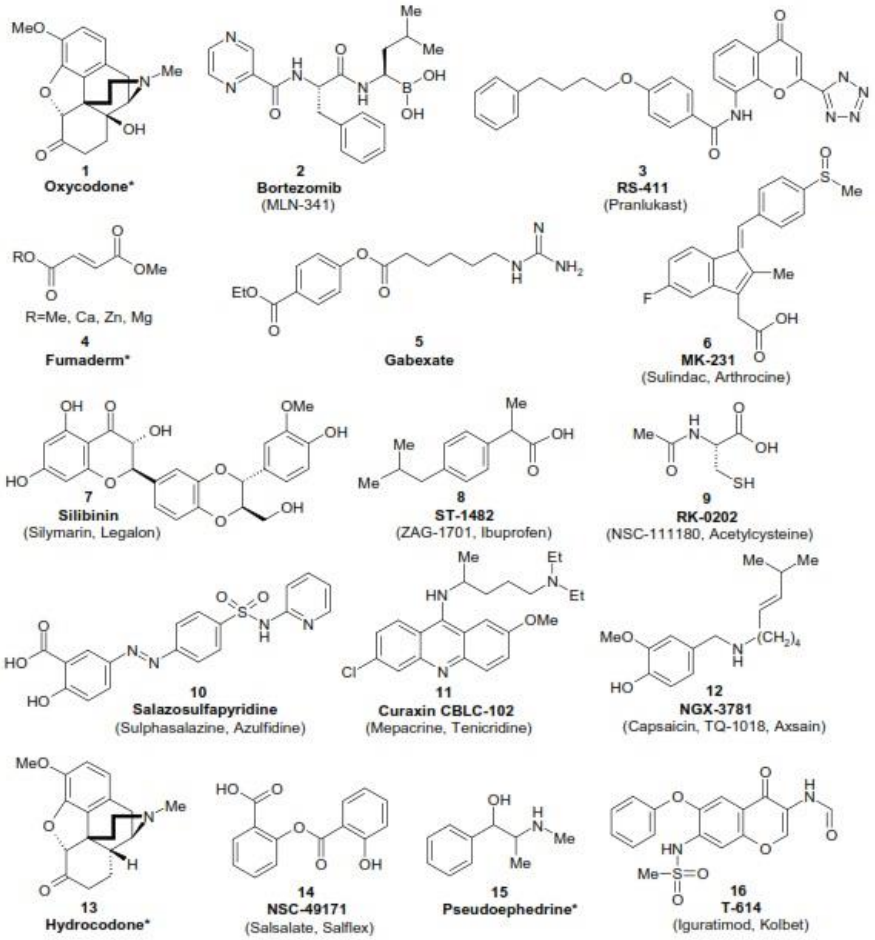


Figure 1. Canonical and alternative NF-κB signaling pathways implicated in inflammation

2. NF-κB Inhibitors

In past decades, enormous resources have been recruited to invent, develop and apply novel therapeutics against inflammation. Among numerous compounds that were found to have considerable physiological and therapeutic significance against different inflammation conditions acting directly on the NF-κB protein complexes or NF-κB-related signaling pathways plant-derived agents (extracts and essence), steroid-based compounds and several small molecule mediators jointly compose a large therapeutic group. Recent advances achieved in different preclinical models have clearly identified a wide therapeutic potential of many small molecular NF-κB inhibitors (Fig. 2), neutralizing antibodies/proteins or genetically altered gene functions against various inflammatory mediators. Several clinically approved drug compounds are currently launched onto the world pharmaceutical market. Table I summarizes a list of large-scale clinical trials that have been conducting by various pharmaceutical firms. Several inhibitors were found to have an improved therapeutic potential then they were used in combination.



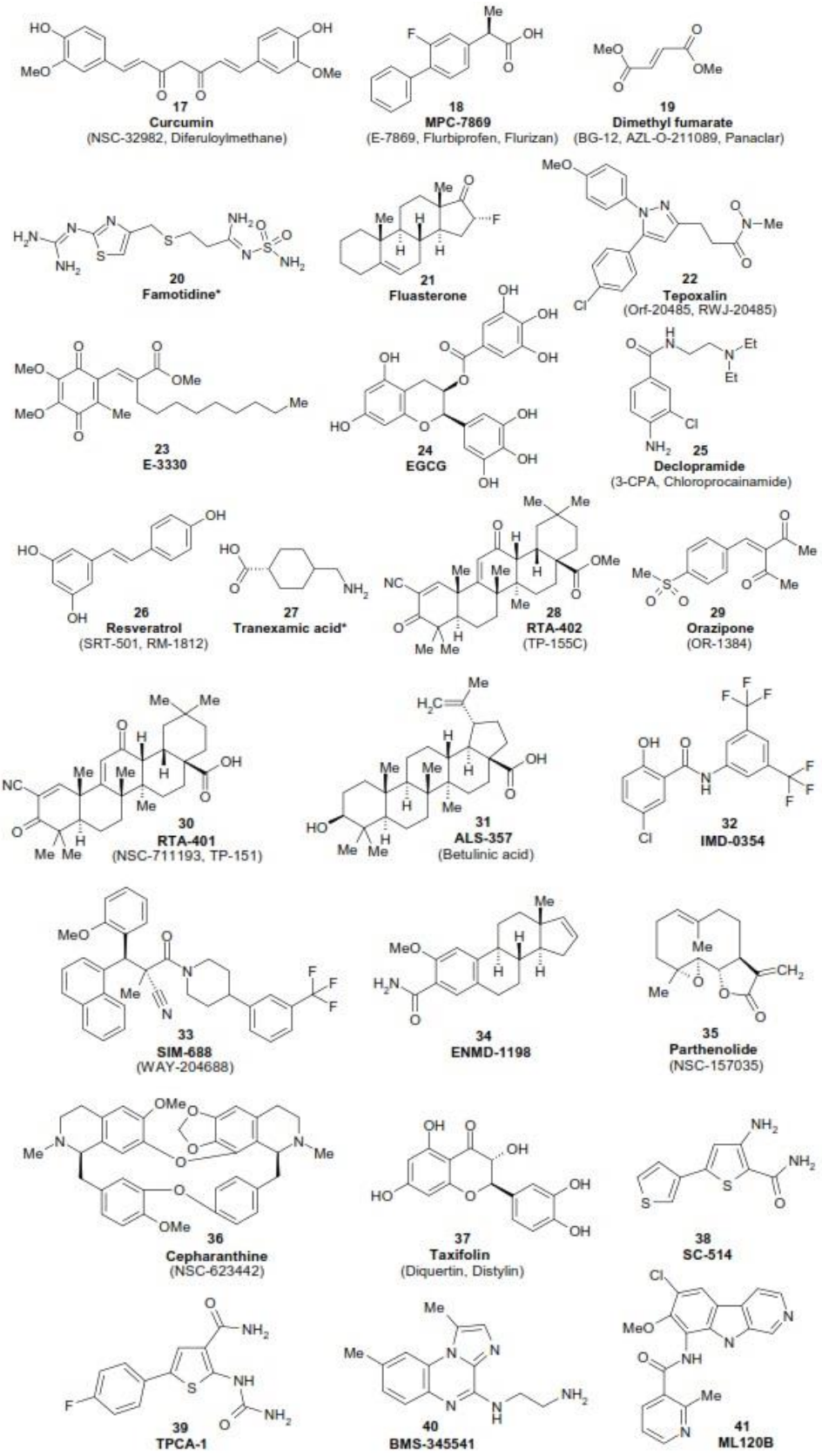


Figure 2. Small molecule NF-κB inhibitors already released onto the pharmaceutical market or currently entered in Phase I-III clinical trials (* Compounds which are commonly used in combination)

Table I. The main characteristics of clinically proven small molecule NF-κB inhibitors

Compound (Fig. 2)	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
1[a]	Launched-2005	Non-specific inflammation	Multi-targeted inhibitor, especially against NF-κB and COX-1/2/3	BTG
2	Launched-2003	Non-specific inflammation and cancer	NF-κB, AP-1 and Proteasome inhibitor	Millennium Pharmaceuticals and Janssen-Cilag
3	Launched-1995	Upper respiratory tract disorders, chronic obstructive pulmonary diseases (COPD), asthma, allergic rhinitis	Multi-targeted inhibitor, especially against NF-κB and Leukotriene CysLT2/ CysLT1	Ono
4 [a]	Launched-1994	Psoriasis and multiple sclerosis	NF-κB targeted inhibitor	Biogen Idec
5	Launched-1978	Pancreatic disorders	Multi-targeted inhibitor, particularly against NF-κB and AP-1	Ono
6	Launched-1976	Ankylosing spondylitis, rheumatoid arthritis, gout, osteoarthritis	NF-κB inhibitor and ABCC1/3 expression enhancer	Merck
7	Launched-1972	Liver and biliary tract disorders, lipoprotein disorders, disorders of the coronary arteries and atherosclerosis, diabetes, viral hepatitis	Multi-targeted inhibitor especially against NF-κB, HMG-CoA reductase, Reverse transcriptase as well as ApoB secretion	Madaus
8	Launched-1969	Ankylosing spondylitis, rheumatoid arthritis, osteoarthritis	NF-κB and COX-1/2/3 inhibitor	Zambon and Merckle GmbH

9	Launched-1968	Renal failure, interstitial lung diseases, inflammatory bowel disease, obsessive compulsive disorder (COPD), metabolic disorders (not specified), psychiatric disorders (not specified), mucolytics, cardiovascular diseases, COPD, cocaine dependency, preterm labor, mucositis	NF-κB targeted inhibitor	Zambon and Yale University
10	Launched-1944	Inflammatory bowel disease and rheumatoid arthritis	NF-κB targeted inhibitor	Pfizer
11	Launched-1932	Prostate and renal cancer therapy, malarials, protozoal diseases, prion diseases	Multi-targeted inhibitor, particularly against NF-κB and Secretory phospholipase A2 (sPLA2)	Bayer
12	Launched	Neuropathic pain and diabetic neuropathy	Multi-targeted inhibitor, especially against NF-κB, TRPV1 and tNOX	NeurogesX
13 [a]	Launched	Non-specific inflammation	NF-κB and COX-1/2/3 inhibitor	Abbott
14	Launched	Rheumatoid arthritis, diabetes, osteoarthritis	NF-κB targeted inhibitor	Roche
15 [a]	Launched	Upper respiratory tract disorders	NF-κB and COX-1/2/3 inhibitor	SCOLR Pharma
16	Pre-Registered	Rheumatoid arthritis	NF-κB targeted inhibitor	Toyama
17	Phase III	Ocular genetic disorders, arthritis, alzheimer's dementia, psoriasis, cystic fibrosis, premalignant conditions, malarials, myelodysplastic syndrome, pancreatic cancer, multiple myeloma, mucositis	Multi-targeted inhibitor, especially against NF-κB, EGFR and CCND1 expression, Glucose-6-phosphatase, HIV Integrase as well as COX-2	Johns Hopkins University

18	Phase III	Non-specific inflammation, arthritis, Alzheimer's dementia, colorectal and prostate cancers, oncolytic disorders	NF-κB modulator and γ-Secretase inhibitor	Loma Linda University and Encore
19	Phase III	Psoriasis and multiple sclerosis	NF-κB targeted inhibitor	Biogen Idec
20 [a]	Phase III	Non-specific inflammation	Multi-targeted inhibitor, especially against NF-κB and COX-1/2/3. Histamine H2 receptor antagonist	Horizon Therapeutics
21	Phase II	Psoriasis, arthritis, systemic lupus erythematosus, diabetes, actinic keratoses, multiple sclerosis, oncolytic disorders	NF-κB targeted inhibitor	Temple University
22	Phase II	Psoriasis, ophthalmic inflammation, allergy and asthma	NF-κB and COX-1/2/3 inhibitor	Ortho-McNeil
23	Phase II	Non-specific inflammation	NF-κB targeted inhibitor	Eisai
24	Phase II	Actinic keratoses, lipoprotein disorders, dermatologic disease, diabetes, ophthalmic disorders, Parkinson's disease, cancers and liver fibrosis	Multi-targeted inhibitor, especially against NF-κB, SGLT-1, PDGFR, BACE, VEGFR, VEGFR-2 (FLK-1/KDR), tNOX, AP-1, etc.	Kyushu University
25	Phase II	Inflammatory bowel disease and colorectal cancer	NF-κB targeted inhibitor	OxiGene
26	Phase II	Psoriasis, ocular disorders, diabetes, disorders of the coronary arteries and atherosclerosis, obesity, herpes virus, neuromuscular genetic disorders	Multi-targeted inhibitor, especially against NF-κB, COX-1, Xanthine Oxidase, MAO-A and BACE1. APOA1 expression enhancer and SIRT1 activator	Royalmount Pharma

27 [a]	Phase II	Non-specific inflammation	NF-κB and COX-1/2/3 inhibitor	Sawai
28	Phase I/II	Inflammatory bowel disease, autoimmune diseases, rheumatoid arthritis, melanoma, solid tumors, renal diseases, pancreatic cancer	Multi-targeted inhibitor, especially against NF-κB, Bcl-2, IKK-1 (IKK-α) and NOX production. PPARγ agonists, NADPH and Heme Oxygenase activator	Dartmouth College and M.D. Anderson Cancer Center
29	Phase I	Inflammatory bowel disease, allergy, asthma	NF-κB targeted inhibitor	Orion Corp.
30	Phase I	Inflammatory bowel disease, stroke, solid tumors, leukemia	Multi-targeted inhibitor, particularly against NF-κB and NOX production. PPARγ agonists	Dartmouth College and National Cancer Institute (US)
31	Phase I	Melanoma and severe acute respiratory syndrome (SARS)	Multi-targeted inhibitor, particularly against NF-κB, DGAT, SARS Coronavirus 3C-like protease and DNA Topoisomerase-I. Caspases 3/8 activator	University of Illinois
32	Phase I	Interstitial lung diseases, disorders of the coronary arteries and atherosclerosis, atopic dermatitis	NF-κB and IKK-2 (IKK-β) inhibitor	Institute of Medicinal Molecular Design
33	Phase I	Rheumatoid arthritis, nonspecific inflammation, sepsis	NF-κB inhibitor and Estrogen receptor (ER) α/β ligand	Wyeth Pharmaceuticals
34	Phase I	Non-specific inflammation and solid tumors	Multi-targeted inhibitor, particularly against NF-κB, STAT-3 and HIF-1α factors	EntreMed
35	Clinical	Atherosclerosis therapy, leishmaniasis, oncolytic disorders, septic shock	NF-κB targeted inhibitor	Ashbury Biologicals
36	Clinical	Non-specific inflammation, HIV infection, cancers	NF-κB targeted inhibitor	Tohoku Pharmaceutical University

37	Clinical	Atherosclerosis, lipoprotein disorders, ischemic stroke, hepatitis virus, HIV infection	Multi-targeted inhibitor, especially against NF-κB, HMG-CoA reductase, Reverse transcriptase and ApoB secretion	Sigma Chemical and National Yang-Ming University
38	Early clinical trials	Non-specific inflammation and anti-tumor promoting effects, particularly against Adenocarcinoma	Selective inhibitor of IKK-2 activity	Pfizer
39	Early clinical trials	Corneal ulcer, COPD and related airway inflammation	Inhibitor of human IKK-2 activity	GlaxoSmithKline
40	Early clinical trials	Lung inflammation including airway inflammation in asthma, arthritis, inflammatory bowel diseases and cancer. It also suppresses graft rejection	Highly selective and potent inhibitor of IKK-2 activity. It bounds to an allosteric binding site	Bristol-Myers Squibb
41	Early clinical trials	Rheumatoid arthritis, COPD (particularly chronic airway inflammation) as well as cancer	Selective, reversible, and ATP-competitive small molecule inhibitor of IKKβ	Millennium Pharmaceuticals

[a] Clinically validated drug combinations: **1**. Combunox (Oxycodone/Ibuprofen); **4**. Fumaderm (Dimethyl fumarate/(Ca,Mg,Zn) Monoethyl fumarates; **13**. Vicoprofen (Hydrocodone/Ibuprofen); **15**. Rhinadvil (Pseudoephedrine/Ibuprofen); **20**. HZT-501 (Famotidine/Ibuprofen); **27**. SMS-113 (Tranexamic acid /Ibuprofen).

3. JAK/STAT Signalling Pathway

The rapidly expanding knowledge underpinning the cytokine transcription factor network has "shed a bright light" on the acute and chronic inflammatory response. JAKs (Janus tyrosine Kinases) and STAT (Signal Transducer and Activator of Transcription) are critical components of many cytokine receptor systems that regulate cell growth, survival, proliferation, hematopoiesis and pathogen resistance. Thus, it was recently uncovered that JAK/STAT signaling network observed in three major cell types involved in inflammatory responses: T-cells, neutrophils, and macrophages, plays a critical role in the pro-inflammatory cytokine production. JAK belongs to a family of non-receptor PTKs comprising of JAK1, JAK2, JAK3 and TYK2 (non-receptor Protein Tyrosine Kinase-

2). STATs are latent cytoplasmic transcription factors that become activated after recruitment to an activated receptor complex. A series of seven separate STAT proteins are present in a wide variety of cell types, including cells of epithelial origin.[vi] These include STAT1 to 6, including STAT5a and STAT5b, which are encoded by distinct genes. For example, STAT-1 mediates a pro-inflammatory response to the activation of the interferon gamma receptor on the cell surface by IF-γ. STAT-3 participates in the signaling pathways for many cytokines in various cells and organs that are regulated by the suppressor of cytokine signaling (SOCS) family, including SOCS3 (see below).[vii] In addition, different isoforms of several STATs have been identified to date. The JAK/STAT signaling pathways are regulated by a vast array of intrinsic and environmental stimuli, which can add plasticity to the response of a cell or tissue.[viii]

Mechanistically, JAK/STAT signaling is relatively simple, with only a few principal components (Fig. 3). A variety of ligands including cytokines, hormones and growth factors, as well as their specific receptors such as cytokine receptors, EGFR, GPCR and INF-Rs activate the JAK/STAT pathway through the multimerization of receptor subunits such as STATIP and Tyk2. Thus, JAK activation occurs upon ligand-mediated receptor multimerization because two JAKs are brought into close proximity, allowing trans-phosphorylation. The activated JAKs subsequently phosphorylate principal targets, including both the receptors and the major substrates, STATs, results in dimerization of STATs through interaction with a conserved SH2 domain. As shown in Fig. 3, different JAKs and STATs are activated by different ligands via corresponding receptors. Phosphorylated STATs dimmers then translocate into the nucleus by a mechanism involving Importin and the Ran nuclear import pathway. In the nucleus, STAT transcriptional complexes bind to specific regulatory regions on DNA to activate or repress transcription of target pro-inflammatory genes. Thus the JAK/STAT cascade provides a direct mechanism to translate an extracellular signal into a transcriptional response. In addition, RTK family that normally regulates the Ras/Raf/MEK/ERK-related signaling cascades including p38 MAPK pathway can also induce the JAK/STAT signal transduction via cytokine or/and interleukin receptors.

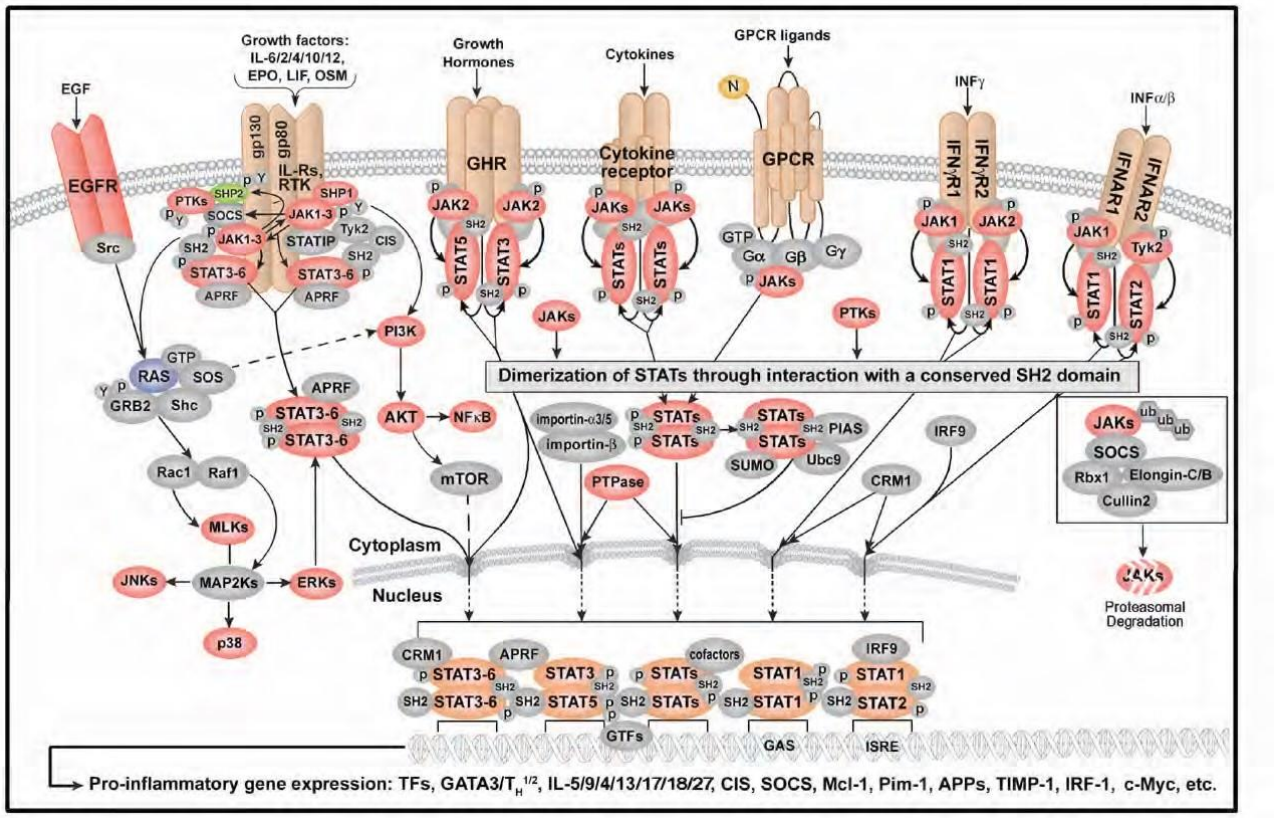


Figure 3. The JAK/STAT signaling pathways implicated in inflammation

3.1. JAK/STAT Inhibitors

There are several small molecule agents that have been shown to regulate JAK/STAT signaling pathway (Fig. 4, Tables II and III). Several compounds are already launched on the market as drugs particularly acting against various inflammatory conditions while others are currently evaluated in different clinical trials.

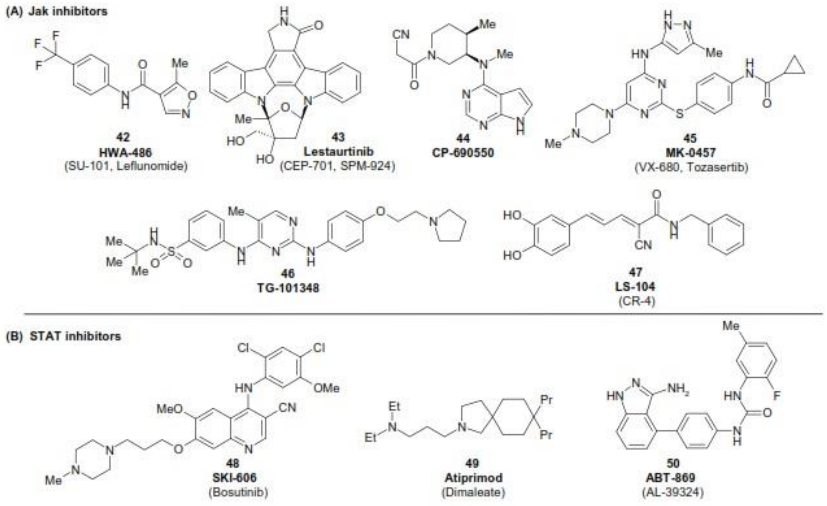


Figure 4. Small molecule agents targeting JAK kinases (**A**) and STAT (**B**) that have already been approved or are currently in clinical trials.

Table II. Therapeutically active small molecule inhibitors of Jak kinases and STAT activities

Compound (Fig. 6)	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
42	Launched-1998	Ovarian, brain and prostate cancers, rheumatoid arthritis, HIV-infection and transplant rejection	Multi-targeted inhibitor, especially against Jak3 kinase and STAT-6	Lepetit and Sanofiaventis
43	Phase II/III	Psoriasis, hematopoiesis, prostate and pancreatic cancers, neurologic cancer as well as myeloid leukemia	Multi-targeted inhibitor, especially against Jak2 kinase, RET, TRKA and Flt3 (FLK2/STK1)	Kyowa Hakko
44	Phase II	Psoriasis, inflammatory bowel disease, rheumatoid arthritis, asthma and transplant rejection	Jak3-targeted inhibitor	Pfizer
45	Phase II	Non-specific and cancerassociated inflammatory diseases	Multi-targeted inhibitor, especially against Aurora-C and Jak2 kinases	Vertex
46	Phase I/II	Cancer and cancer-associated inflammatory diseases	Jak2 and Flt3 (FLK2/STK1) inhibitor	TargeGen
47	Phase I	Cancer-associated inflammatory diseases	Jak2, Bcr-Abl and Flt3 (FLK2/STK1) inhibitor	Hospital for Sick Children
48	Phase III	Cancer and cancer-associated inflammatory diseases, ischemic stroke	Multi-targeted inhibitor, particularly against STAT-5 and Src, Bcr-Abl, Abl kinases	Wyeth Pharmaceuticals
49	Phase II	Bone resorption, endocrine and colorectal cancers, multiple myeloma, inflammatory bowel disease and rheumatoid arthritis	Multi-targeted inhibitor, particularly against STAT-3 (IL-6 production inhibitor) and PKB/Akt Apoptosis inducer (Caspase-3/9 activator)	AnorMED and GlaxoSmithKline
50	Phase II	Cancer and cancer-associated inflammatory diseases	Multi-targeted inhibitor, particularly against STAT-5, ERK, CSF1R (c-FMS), PDGFRβ, VEGFR-2 (FLK1/KDR), Flt3 (FLK2/STK1)	Abbott

Table III. Small molecule inhibitors of Jak kinase and STAT which structures are not disclosed yet.

Compound Name(s)	Development Phase	Therapeutic Targets	Mechanism of Action	Originator(s)
INCB-018424 (INCB-18424)	Phase II	Psoriasis, thrombocytopenic anemia, hematological and prostate cancers, multiple myeloma, rheumatoid arthritis as well as PMF [a] and POST-PV/ET MF [a]	Jak2-targeted inhibitor	Incyte
AT-9283	Phase I/II	Cancer-associated inflammatory diseases	Multi-targeted inhibitor, especially against Jak2, Aurora-A/B, Bcr-Abl kinases	Astex
R-348	Phase I	Psoriasis, autoimmune diseases, multiple sclerosis, rheumatoid arthritis and transplant rejection	Jak2-targeted inhibitor	Rigel
XL-019	Phase I	Non-specific inflammation, hematopoiesis and oncolytic diseases	Jak2-targeted inhibitor	Exelixis

[a] PMF - Primary Myelofibrosis; Post-PV/ET MF - Post Polycythemia Vera/Essential Thrombocythemia Myelofibrosis.

For example, Leflunomide **42** is widely used to treat rheumatoid arthritis and fibrosis, Lestaurtinib **43** and MK-0457 **45** are being studied in a Phase II/III clinical trial in patients with treatment-resistant chronic myelogenous leukemia (CML), or Philadelphia chromosome-positive acute lymphoblastic leukemia. CP-690550 **44**, a novel, selective small molecule inhibitor of JAK3 kinase activity with promising anti-inflammatory potential, while TG-101348 **46**, a potent, selective, and orally bioavailable inhibitor of JAK2 is currently in clinical trials against myeloproliferative and inflammatory diseases,[ix] etc.

Concept and Applications

AI-library design at CDL involves:

• *A combined profiling methodology that provides a consensus score and decision based on various advanced computational tools*:

1. Bioisosteric morphing and funneling procedures in designing novel potential AI-agents with high IP value. We apply CDL's proprietary Chemosoft™ software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.
2. Kohonen Self-organizing Maps as a strategic approach to AI-library profiling.
3. A molecular docking approach to the focused library design.
4. Computational-based `*in silico*` ADME/Tox assessment for novel compounds includes prediction of human CYP P450-mediated metabolism and toxicity as well as many pharmacokinetic parameters, such as Brain-Blood Barrier (BBB) permeability, Human Intestinal Absorption (HIA), Plasma Protein binding (PPB), Plasma half-life time ($T_{1/2}$), Volume of distribution in human plasma (V_d), etc.

The fundamentals for these applications are described in a series of our recent articles on the design of exploratory small molecule chemistry for bioscreening [for related data visit ChemDiv. Inc. online source: www.chemdiv.com].

• *Synthesis, biological evaluation and SAR study for the selected structures*:

1. High-throughput synthesis with multiple parallel library validation. Synthetic protocols, building blocks and chemical strategies are available.
2. Library activity validation via bioscreening; SAR is implemented in the next library generation.

We practice a multi-step approach for building our AI-library:

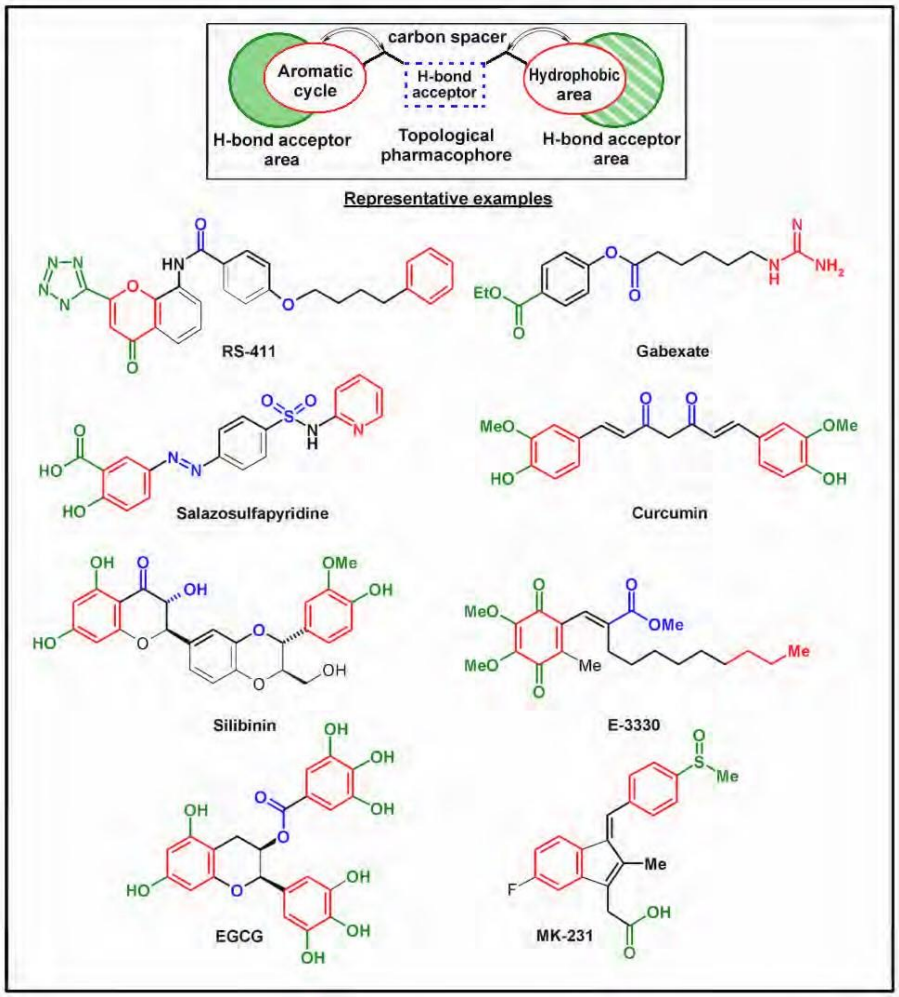
Virtual screening

High-throughput screening of large diversity-based libraries still remains a common strategy within many pharmaceutical companies for the discovery of novel anti-inflammatory agents. However, as noted by many researchers in the field, there is no evidence that high-throughput technologies, including parallel synthesis/combinatorial chemistry and HTS provided the expected impedance to the lead discovery process. Therefore, a number of approaches have been used for the design of more focused screening libraries. These range from pharmacophore and target structure-based design through combinatorial approaches to various QSAR methods. Thus, we have used some of the mentioned *in silico* strategies to design our AI-library. In particular, we have disclosed, how the knowledge obtained from receptor-ligand interaction models and structures of known ligands can be

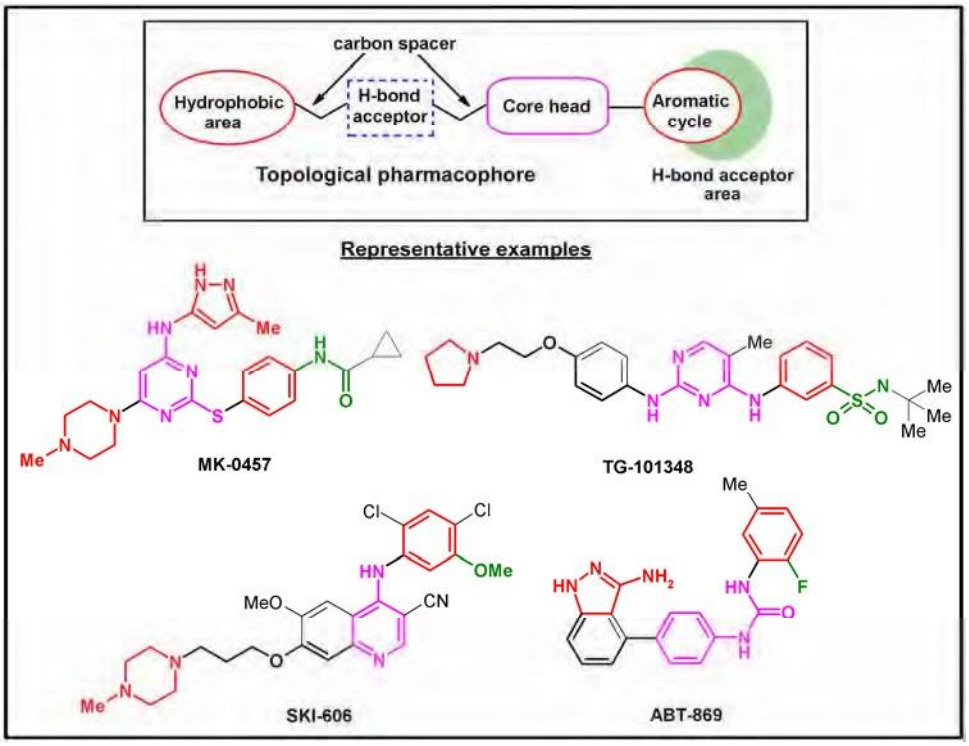
applied for the design of pharmaceutically relevant small-molecule inhibitors of NF-κB and JAK/STAT signaling pathways.

Topological pharmacophores analysis & bioisosteric morphing

In general, bioisosteric transformation allows for a better balancing between different lead-like parameters including specificity, physicochemical and PKPD properties. In addition, this approach provides insight into the patentability of lead candidates. Structural transformations occurred largely among a wide range of synthetic compounds are often based on bioisosteric modifications and specific topological skeleton of naturally derived compounds. Bioisosteric morphing provides a solid foundation for analysis of key structural elements which can further be combined in common topological pharmacophore. Typical examples of key bioisosteric modifications clearly observed among NF-kB and JAK/STAT inhibitors as well as topological pharmacophore are shown in Fig. 5. The modified structural fragments are highlighted in color.



(A)



(**B**)

Figure 5. A common topological pharmacophore and bioisosteric transformations of NF-kB (**A**) and JAK/STAT (**B**) inhibitors

As shown in Fig. 5, many of NF-kB and JAK/STAT inhibitors belonging to different structural classes commonly contain several key structural motifs. For example, in the case of NF-kB inhibitors these include two H-bond acceptor areas (green), one hydrophobic and one aromatic area or two aromatic areas (red), carbon spacer (black), which can also be amplified by H-bond acceptor (blue) or double bonds. In turn, many of JAK/STAT inhibitors share the same topological elements with minor differences (e.g., two aromatic and/or one H-bond acceptor areas can be safely ignored). The "core head" includes pyrimidine fragment or its bioisosteric modifications such as pyridine (SKI-606) or various linear chains which are direct analogues of the core heterocyclic head (ABT-869). Structural elements which are not regarded as significant in the core topological pharmacophore are highlighted by dotted lines. The classical bioisosteric transformations (e.g., cyclic analogues of linear molecules, carboxyl group/tetrazole, carbocycle/heterocycle or carboxamide/sulfonamide) which can frequently be found in many medicinal chemistry studies are clearly shown by the example of RS-411, E-3330 and Salazosulfapyridine, Silibinin and Curcumin as well as TG-101348, SKI-606 and ABT-869. The mirror composition of carbon spacer and hydrophobic cap was featured in several NF-kB inhibitors for example, as in the pair of Pseudoephedrine and MPC-7869. Double bounds sited intermittently

along the carbon spacer provide a rigid conformational state which, in a number of cases, is strongly correlated with activity and/or selectivity of drug compounds, for example, for Curcumin and Resvertanol.

Self-organizing Kohonen maps

Self-organizing Kohonen maps belong to a class of neural networks known as competitive learning or self-organizing networks which in turn are based on unsupervised learning rule. They were originally developed to model the ability of the brain to store complex information as a reduced set of salient facts without loss of information about their interrelationships. High-dimensional data are mapped onto a two-dimensional rectangular or hexagonal lattice of neurons in such a way as to preserve the topology of the original space. This methodology has successfully been used in various medicinal chemistry applications.

We have used this approach for compound selection and focused-library profiling. Initially, we have collected a 22,110-compound database of known drugs and compounds entered into preclinical or clinical trials; their structures and assignments were obtained from Prous Science Integrity [Prous Science, URL: http://www.prous.com]. Each compound within this database was characterized by a defined profile of target-specific activity, focused against 1 of more than 100 different protein targets. In particular, this set included more than 473 NF-kB- and JAK/STAT-targeted agents which were shown to have activity against theses targets; representative structures are shown in Figs. 2 and 4. The whole dataset was then filtered and preprocessed. It was filtered based on MW (not more than 800). Molecular features encoding the relevant physicochemical and topological properties of compounds were then calculated using SmartMining software [URL: http://www.ChemDiv.com] and selected by PCA. These molecular descriptors encode the most significant molecular features, such as molecular size, lipophilicity, H-binding capacity, flexibility, and molecular topology. As a result of specific selection procedure, at the output, an experimental set consisted of 7 molecular descriptors including Zagreb index, E-state indexes for the following structural fragments: >C-, -CH_2-, -CH_3, the number of H-bond donors, HB2 (a structural descriptor which encodes the strength of H-bond acceptors following an empirical rule) and LogP was determined. This set was then used for Kohonen map generation. Taken in combination, they define both pharmacokinetic and pharmacodynamic behavior of compounds and are effective for property-based classification of target-specific groups of active agents. However, it should be noted that for each particular target-specific activity group, another, more optimal set of descriptors can be found, which provides better classification ability.

A Kohonen SOM of 22K pharmaceutical leads and drugs generated as a result of the unsupervised learning procedure is depicted in Fig. 6. It shows that the studied compounds occupy a wide area on the map, which can be characterized as the area of druglikeness. Distribution of various target-specific groups of ligands in the Kohonen map demonstrates that most of these groups have distinct locations in specific regions of the map (Fig. 7a-l). In particular, as shown in Figs 7d,e, pharmaceutically relevant agents targeted against NF-κB and JAK/STAT occupy compact/distinct areas within the map constructed. The classification accuracy of Kohonen modeling was approx. 82% for NFκB and 78% for JAK/STAT inhibitors. It is a statistically relevant prediction quality therefore this model can be effectively used for virtual library profiling of the current interest.

A possible explanation of these differences is in that, as a rule, receptors of one type share a structurally conserved ligand-binding site. The structure of this site determines molecular properties that a receptor-selective ligand should possess to properly bind the site. These properties include specific spatial, lipophilic, and H-binding parameters, as well as other features influencing the pharmacodynamic characteristics. Therefore, every group of active ligand molecules can be characterized by a unique combination of physicochemical parameters differentiating it from other target-specific groups of ligands. Another explanation of the observed phenomenon can be related to different pharmacokinetic requirements to drugs acting on different biotargets.

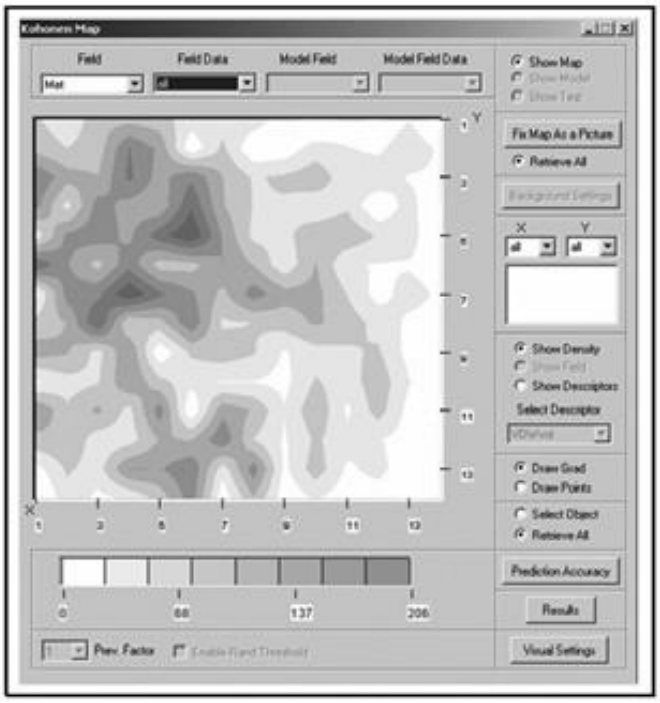


Fig. 6. Property space of 22K pharmaceutical leads and drugs visualized using the Kohonen map. The data have been smoothed

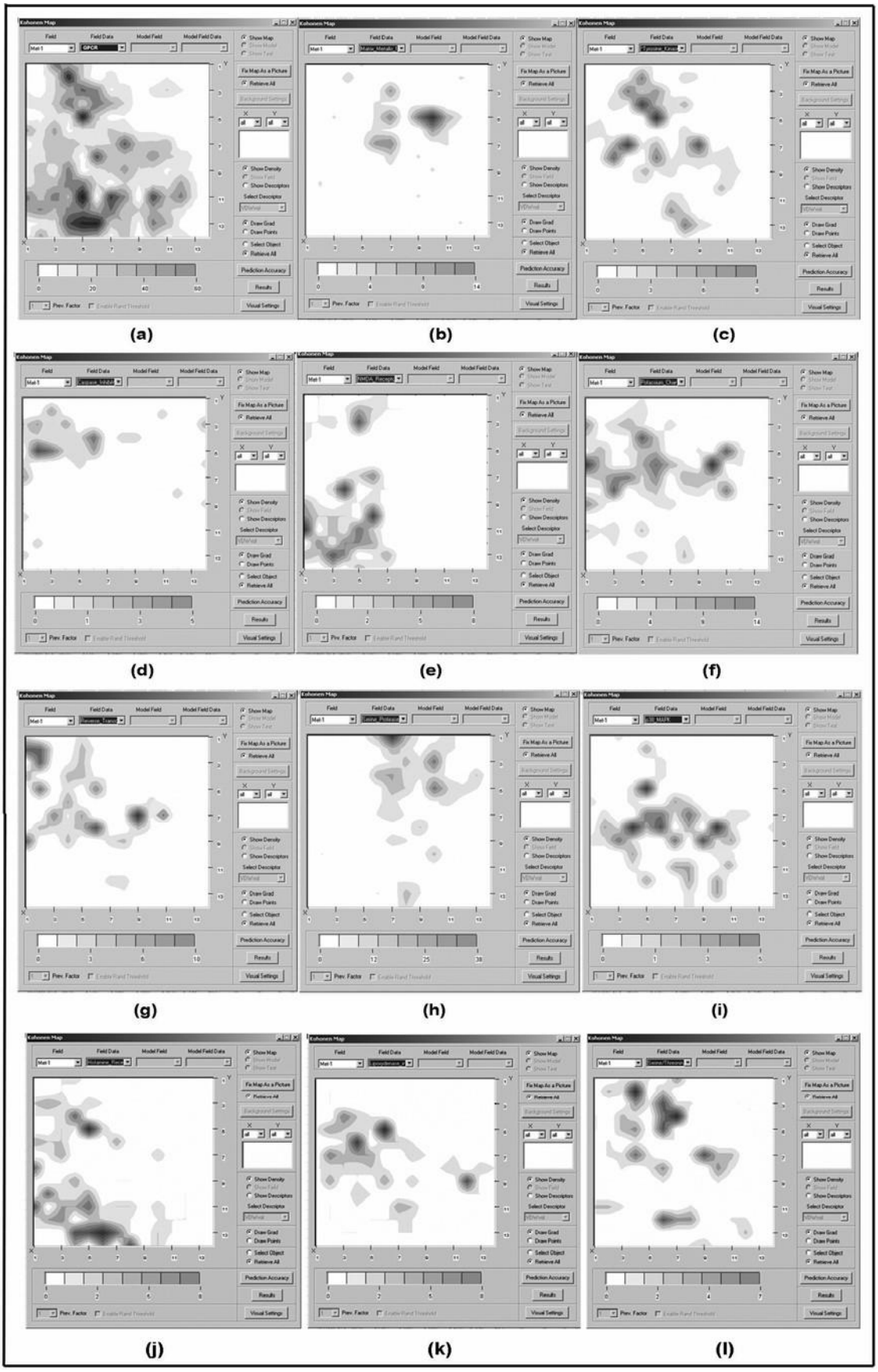

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)

Fig. 7. Distribution of nine large target-specific groups of pharmaceutical agents within the Kohonen map: (a) GPCR agonists/antagonists (5432 compounds); (b) matrix metalloproteinase inhibitors (120 compounds); (c) tyrosine kinase inhibitors (175 compounds); (d) JAK/STAT inhibitors (195 compounds); (e) NF-κB inhibitors (278 compounds); (f) potassium channel blockers/activators (302 compounds); (g) reverse transcriptase inhibitors (160 compounds); (h) serine protease inhibitors (531 compounds); (i) p38 MAPK inhibitors (100 compounds); (j) histamine receptor antagonists (168 compounds); (k) lipoxygenase inhibitors (114 compounds); (l) serine/threonine kinese inhibitors (120 compounds)

The described algorithm is quite effective tool for synthesis planning of *de novo* chemical libraries. Due to a series of specific filters, the properties of a virtual chemical space to be synthesized can be modulated in a wide range of possibilities in order to optimize them according to the purposes of a particular bioscreening program. Usually, the practical design of target-specific combinatorial libraries also includes elements of other virtual sreening approaches, such as selection by structural similarity to known selective ligands (including bioisosteric, topologic, heterocyclic, and substructure similarity), 3D pharmacophore search, flexible docking, etc. After synthetic feasibility assessment, the combinatorial libraries focused towards particular biotargets are synthesized and used in primary screenings. This general strategy is applicable for generating the focused libraries towards several protein target classes, such as GPCRs, protein kinases, nuclear receptors and ion channels. Thus, using the constructed Kohonen map, we have tested an internal set of diverse representative compounds obtained from ChemDiv chemical database (these libraries are available as commercial products at ChemDiv, Inc.).

Initially, a set of compounds consisted of 50K structures of high diversity was exported from ChemDiv database as an SDF-file with a unique ID-number per each structure. Subsequently, it was imported into the SmartMining software (http://www.ChemDiv.com), thus the experimental internal database was successfully formed. Just after the import stage was finished, the previously saved neural model was loaded and the appropriate descriptors were automatically calculated. After the descriptor calculation procedure was completed, the location of the tested structures was determined using the Kohonen algorithm. The corresponding maps are shown in Fig. 8a-c.

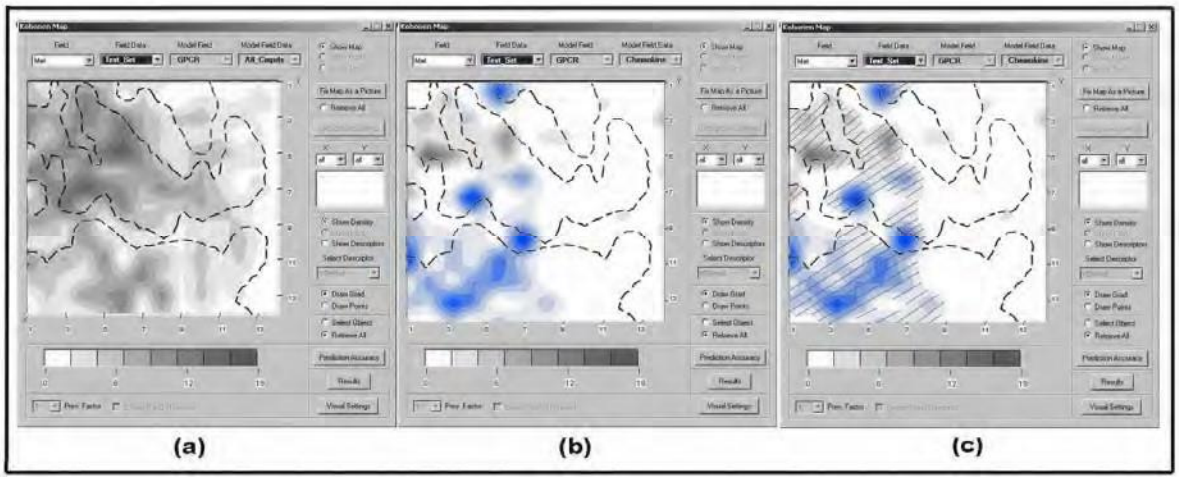


Fig. 8. Distribution of the tested compounds (dotted line) within the Kohonen map: (a) the overlapping with a whole pharmaceutical area; (b) the overlapping with NF-kB (blue) and JAK/STAT (grey) inhibitors areas; (c) the selection of compounds which can be regarded as potential agents acting against NF-kB and JAK/STAT (shaded area)

The `high-score` compounds which were formally classified by the algorithm as potential NF-kB agents (4K structures) as well as JAK/STAT inhibitors (5K compounds) were then exported into an external SDF-file. As a result, a total of 9K compounds were selected and then evaluated through the docking study described below.

Target-based Design

Several actual 3D-QSAR studies were focused for NF-κB and JAK/STAT structure identification and binding sites determination.[x] In particular, the classical target-based design using molecular docking approach was recently carried out to provide a basis for the development of novel small molecule inhibitors of these signaling pathways.[xi] Thus, unique 3D-docking models for NF-κB and JAK/STAT proteins have been developed based on crystallographic data (these data were obtained from: http://www.rcsb.org) and on the data obtained from scientific literature. Using the 3D-models constructed we have selected more than 1500 `high-score` compounds into our AI-library. Representative results of the docking evaluation are presented in Figs. 9-12.

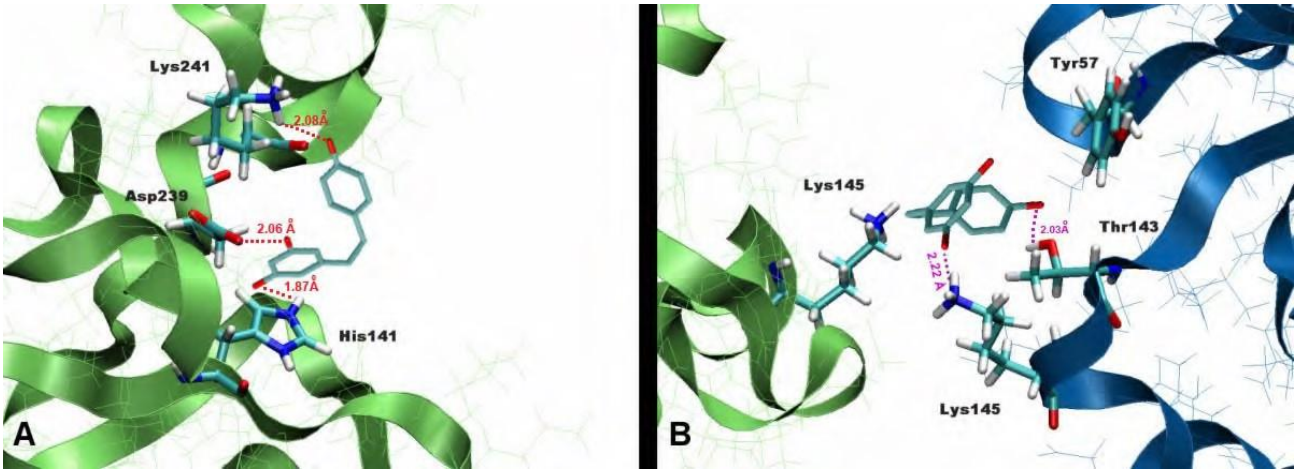


Fig. 9. Binding modes of Resveratrol (**26**) docked into the active site of NF-κB p50: (**A**) monomer and (**B**) homodimer (chain A, shown in green). The residues involved in the interaction with the ligand are shown; the hydrogen bonding and the relative distances are indicated in purple. This model we have used as the `template` binding model

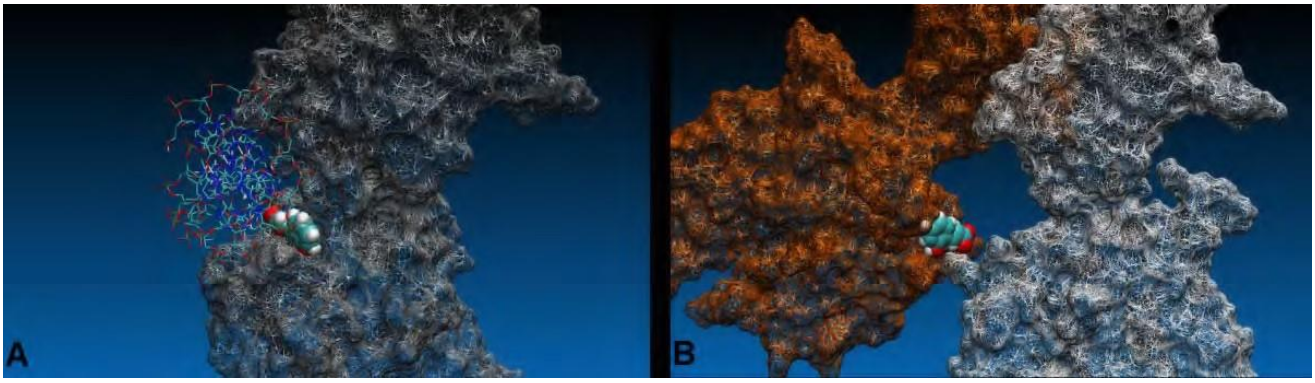
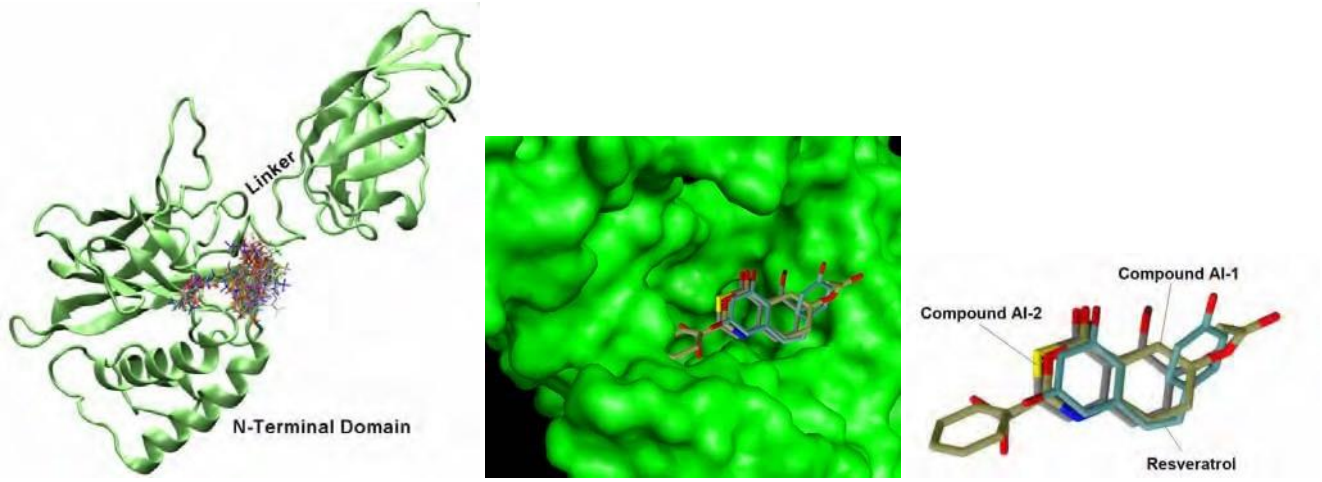


Fig. 10. Poses of docked Resveratrol (**26**) into the DNA binding region of NF-κB p50. (**A**) monomer and (**B**) homodimer (chain A, shown in green). The inhibitory activity of Resveratrol may be due to its ability to form a stable complex with the active conformation of the dimer and/or blocking the interaction of DNA with the monomer filling the protein binding site. The DNA was obtained from the crystal structure of the homodimer NF-kappaB. The surface of the protein is represented in wire frame, the ligand and DNA are highlighted in VDW and stick representation, respectively



(**A**) (**B**) (**C**)

Fig. 11. (**A**) a stereoview of representative compounds form ChemDiv NF-κB-targeted library docked in to DNA binding region of the NF-κB p50 monomer chain A (the macromolecule is highlighted in green); (**B**) Resvertanol (**26**) and two structurally related compounds AI-1 and AI-2 form AI-library docked into the DNA binding site of the protein; (**C**) the overlapping between Resvertanol (**26**) and the tested compounds

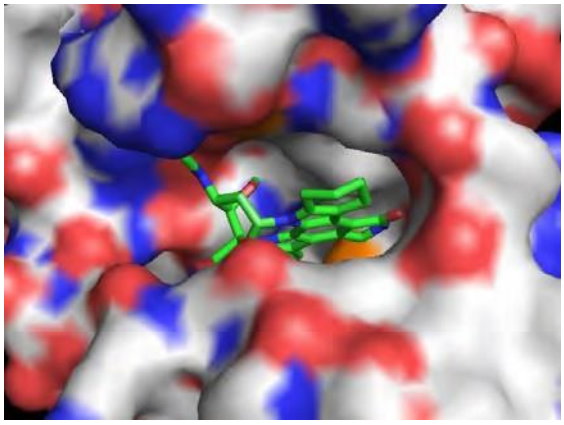


Fig. 12. Crystal structure of the Jak3 kinase domain in complex with AFN 941 (Staurosporine analogue) (**43**)

We have effectively used all the strategies described to design our internal AI-library with the prime focus on the structure-based design.

Synthesis and biological evaluation

(4) Novel AI-libraries are synthesized according to the above criteria.

(5) The subsets of AI-library are validated by bioscreening in collaboration with academic institutions.

Our strategy has proven to be efficient for generation of protein class-targeted libraries. The higher hit rate over diverse libraries, along with identification of novel active chemotypes with optimized diversity and ADME properties, has been shown in multiple studies. Using the computational approaches listed above we have compiled AI-library consisted of more than 9K small molecule compounds Representative set of AI-biased compounds is shown below.

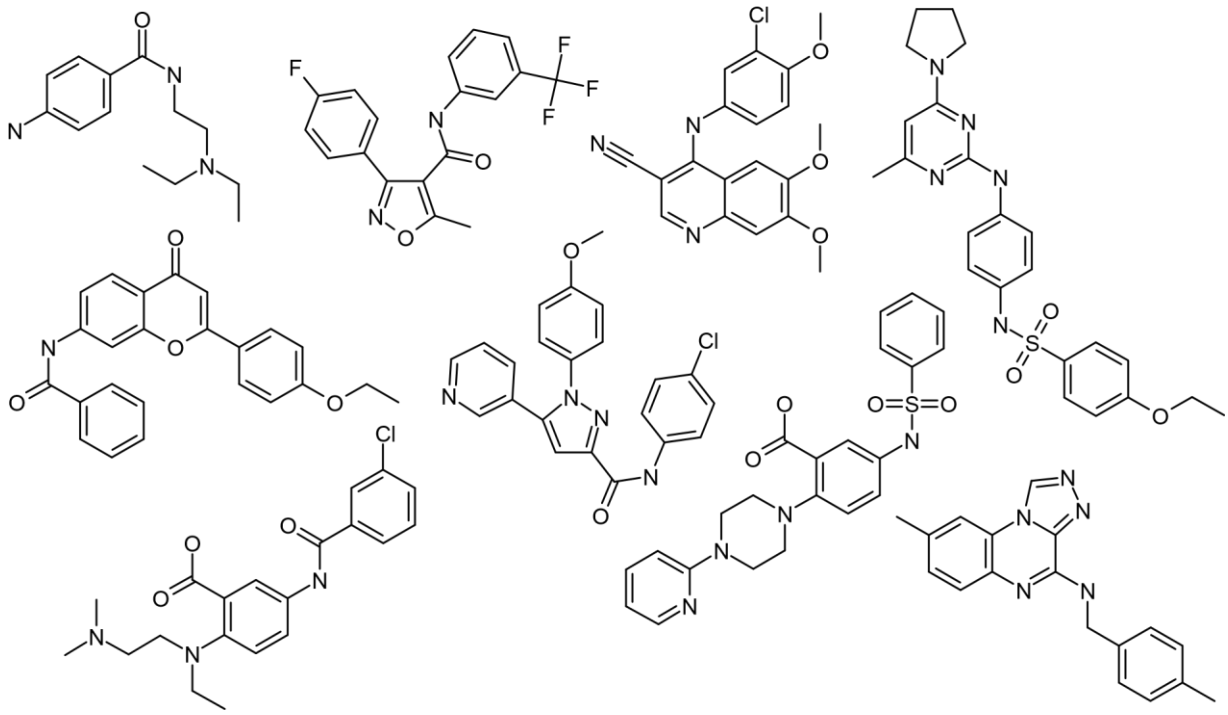


Figure 13. Examples of compounds from the AI-library targeted against NF-κB and JAK/STAT signal transduction proteins

Conclusion

It became abundantly clear that a spectrum of inflammatory disorders include various diseases that tightly coupled with uncontrolled expression of different pro-inflammatory factors, such as cytokines and chemokines, growth factors and various immune response regulators. Their production and activity are, in turn, controlled precisely by different signaling systems including COX-2, NF-κB, MAPK, JAK/STAT, etc. Because of significant side effects currently revealed and elucidated for many COX-2 inhibitors, the novel small molecule regulators of alternative NF-κB and JAK/STAT signaling cascades as well as related precursor molecules have already received a great deal of attention as promising drug candidates for the treatment of various inflammatory conditions, including rheumatoid arthritis, psoriasis, multiple sclerosis, COPD and diabetes. To date, many of these

compounds have already launched in the market while others are currently being evaluated in different clinical trials in the hope of developing novel, effective, and at the same time safe therapeutics.

In the present description we have developed an effective *in silico* approach for targeted library design. It principally based on the unique multi-step procedure that includes the following key stages: topological pharmacophores analysis and bioisosteric morphing, Kohonen-based Self-organizing mapping as well as 3D-molecular docking study. As a result, ChemDiv AI-focused library includes more than 8K `high-score` small molecule compounds specifically targeted against NF-kB and JAK/STAT signaling pathways. This library is updated quarterly based on a "cache" principle. Older scaffolds/compounds are replaced by templates resulting from our in-house development (unique chemistry, literature data, computational approaches) while the overall size of the library remains the same (ca. 9K compounds). As a result, the library is renewed each year, proprietary compounds comprising 50-75% of the entire set. Clients are invited to participate in the template selection process prior to launch of our synthetic effort.

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