NF-κB-targeted library

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1. Introduction

Many of the pro-inflammatory mediators involved in various inflammation diseases as well as cancer have a common feature: their expression in microglial cells is primarily regulated by NF-κB. The transcription factor NF-κB, first described by David Baltimore's group in 1986 as a transcription factor which is essential for the expression of mouse kappa light chain genes [1], has now been found to control gene expression of many of proinflammatory responses. NF-κB is a "master switch" for inflammatory gene expression [2]. Inflammatory cytokines such as TNF and IL-1α and β, bacterial products such as lipopolysaccharide (LPS), and products of cellular damage strongly activate inflammatory responses through the activation of NF-κB. NFκB subsequently plays an essential role in the inflammatory response through regulation of genes encoding inflammatory cytokines (IL-1β, TNFα, IL-12/23), chemokines (IL-8, MIP-1α, MCP-1 [3]), nitric oxide production (iNOS), NADPH oxidase subunits p47 and p67 [4], and adhesion molecules (ICAM-1, VCAM, and E-selectin [5]). Activation of NF-κB is a key event in many chronic inflammatory diseases such as asthma, cardiovascular disease [6], tissue reperfusion injury [7], experimental autoimmune encephalomyelitis (EAE) [8], rheumatoid arthritis [9], and inflammatory bowel disease (IBD) [10]. Many of the standard agents used to treat human inflammatory conditions, including sulfasalazine, 5-aminosalicylates, and corticosteroids, as well as some natural anti-inflammatory compounds such as IL-10, TGFβ1, β2AR agonists, glutamate, and curcumin, among others, have been postulated to exert some of their anti-inflammatory effects through NF-κB inhibition [11]. These compounds are potent inhibitors of microglial activation and are neuroprotective to dopaminergic neurons *in vitro* and/or *in vivo*. Thus, NF-κB activity emerges as a key target to control the chronic inflammation in humans, cancer progression, and strategies for its use in Parkinson's disease to inhibit NF-κB activity in microglial cells more potently may lead to more effective treatments for this disease.

The NF-κB family consists of dimeric transcription factors which include five members: c-Rel, RelA (p65), RelB, NF-κB1 (p50/p105), and NF-κB2 (p52/p100). There are two major pathways of activation: the classical or canonical pathway and the alternate or noncanonical pathway. The classical pathway, which is thought to regulate the production of most pro-inflammatory mediators, is mediated through the activation of a dimer of Rel proteins p50 and p65, complexed within the cytosol to the inhibitory complex IκBα (*see below*). The activation of the classical NF-κB pathway is dependent on the phosphorylation, ubiquitination, and subsequent proteasome-dependent degradation of IκBα. The

phosphorylation of IκBα on serine residues is mediated by IκB kinase (IKK), which is a molecular complex of three proteins consisting of a heterodimer of the two catalytic units IKKα and IKKβ, along with IKKγ (the NF-κB essential modulator, NEMO) [12]. Mice null for IKKβ, IKKγ, or p65 but not IKKα are embryonic lethal as a result of massive liver apoptosis. Cells derived from these embryos are unresponsive to classical NF-κB inducers such as TNFα and IL-1β [13], demonstrating a signaling link between p65, IKKβ, and IKKγ subunits. Activation of the IKK in response to inflammatory mediators like TNFα, IL-1β, and LPS depends critically on the presence of the IKKγ (NEMO) subunit of the IKK complex [14] and results in the phosphorylation of the IκB by the kinase activity of IKKβ [15]. An N-terminal region of NEMO associates with a hexapeptide sequence within the C-terminus of both IKKα and IKKβ, named the NEMO-binding domain (NBD), and disruption or mutation of this NEMO-NBD interaction site on either IKKβ or IKKγ results in a loss of responsiveness of cells to pro-inflammatory signaling.

On the other hand, the non-canonical pathway of NF-κB consists of heterodimers of Rel proteins p100/RelB that also have transcriptional activity but appear to play more of a regulatory role in cellular activation and differentiation rather than in inflammation. In response to a set of factors that include CD40L, B cell-activating factor, and lymphotoxin-β, NF-κB is activated through an alternative pathway independent of IKK [16]. Instead, activation proceeds through the NF-κB-inducing kinase (NIK) that phosphorylates and activates IKKαα homodimers which, in turn, phosphorylate p100 in complex with RelB. This leads to ubiquitin-dependent processing of p100 to p52 and translocation of p52/RelB to the nucleus [17]. Cytokine-induced activation of the noncanonical pathway of NF-κB is accompanied by an increase in the concentration of nuclear IKKα that phosphorylates histone H3 [18]. In cells exposed to cytokines, nuclear IKKα regulates gene expression through promoter-associated histone phosphorylation and binding to promoter regions of NF-κB responsive genes. Mice deficient in IKKα die perinatally, with phenotypical changes of dermal and skeletal development [19]. B-cell activating factor, NIK, and p100/p52 knockout mice have similar phenotypes [20], suggesting that these molecules are all part of the same linear nonclassical signaling cascade. In addition, the classical and alternative pathways are thought to regulate distinct genes in response to their various activators [21].

2. 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.[22] 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.[23] 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[24] 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.[25] A schematic diagram of the canonical and alternative NF-κB pathways is shown in Fig. 1.

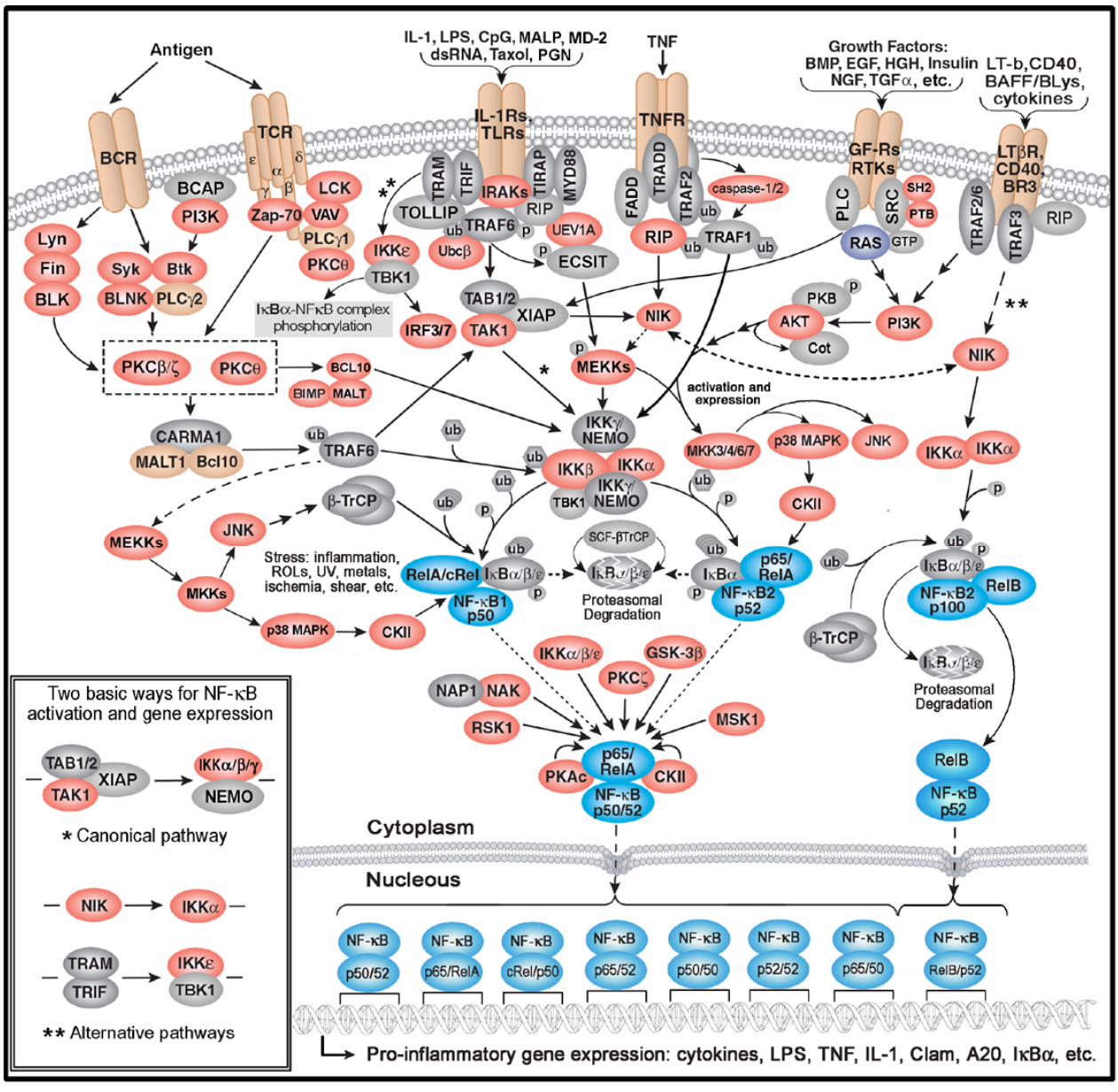
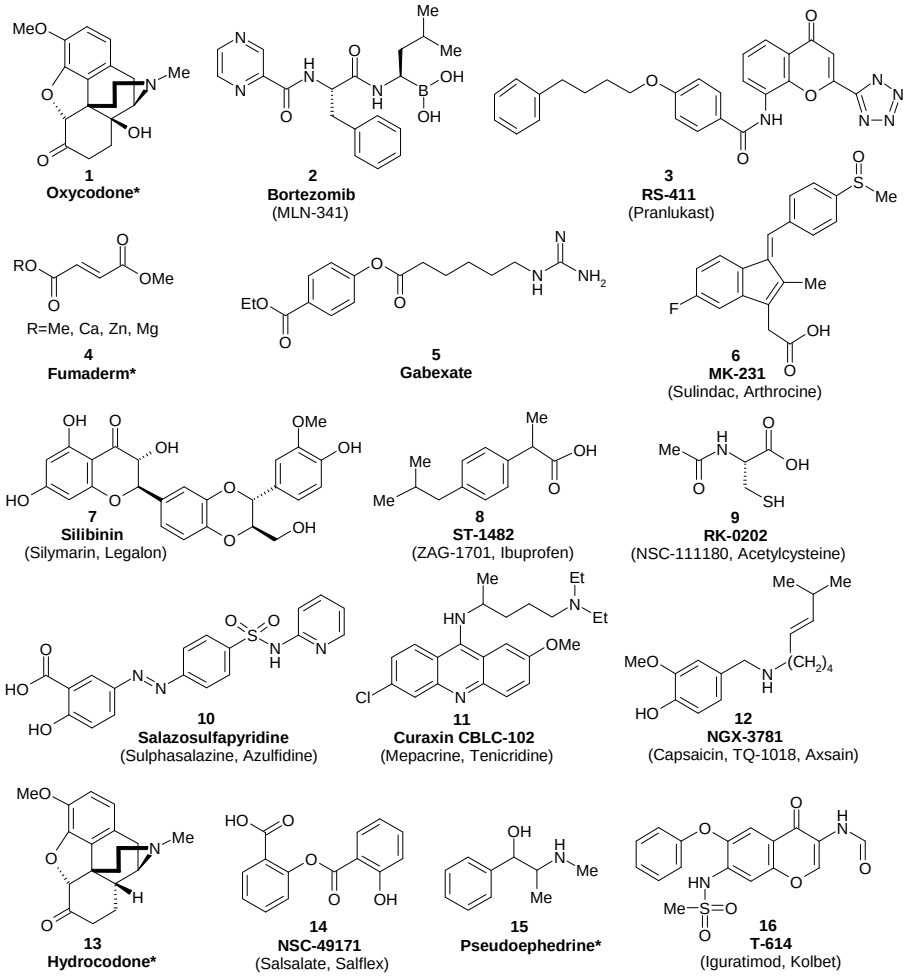


Figure 1. Canonical and alternative NF-κB signaling pathways implicated in inflammation

2.1. 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.



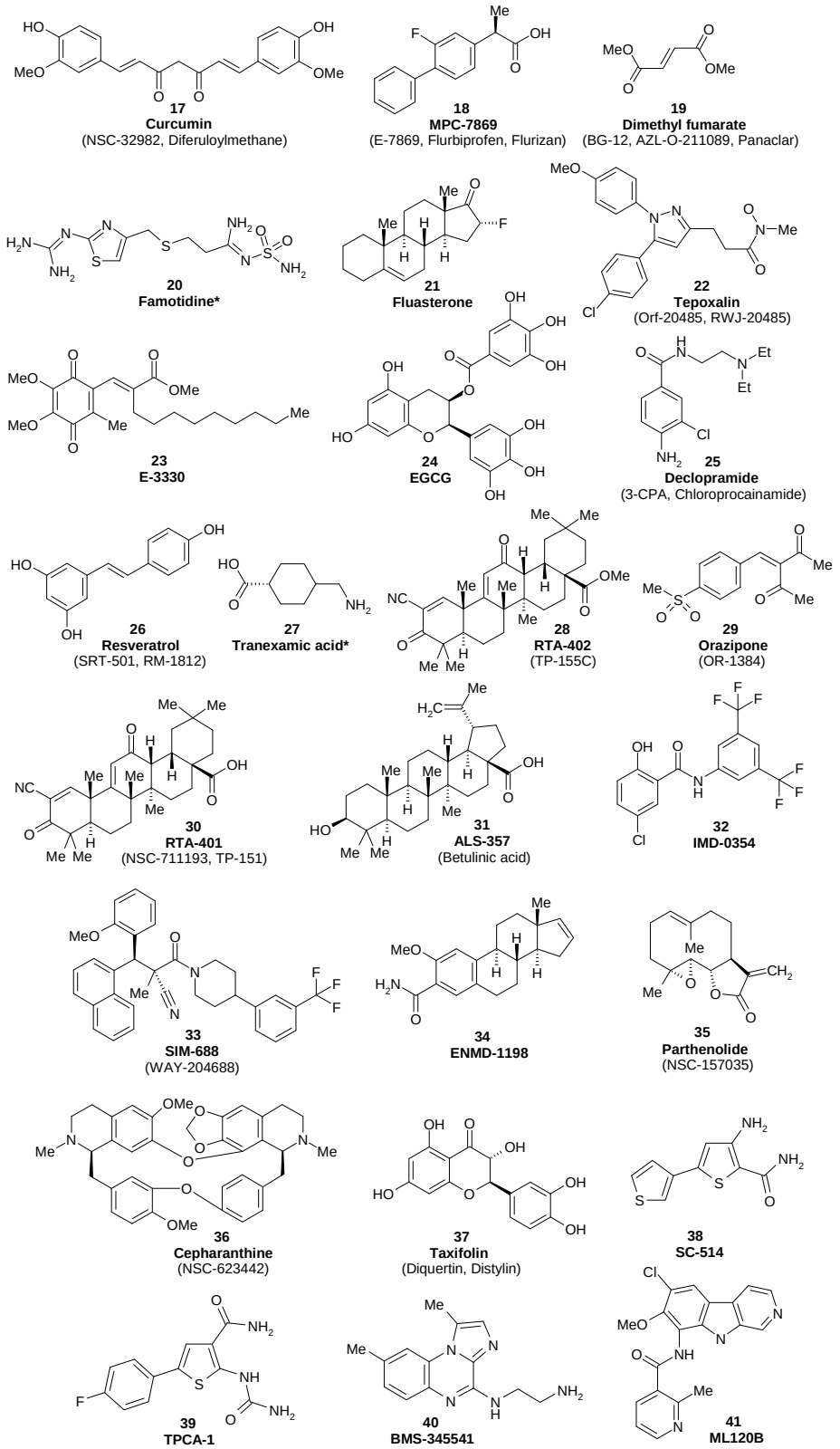


Figure 2. Small molecule NF-κB inhibitors already released onto the pharmaceutical market or currently entered in Phase I-III clinical trials (* Compounds which are commonly used in combination)

Table 1. 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	BTG

			COX-1/2/3	
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	Multi-targeted inhibitor, especially against NF-κB, SGLT-1, PDGFR, BACE, VEGFR, VEGFR-2 (FLK-	Kyushu University

		disorders, parkinson disease, cancers and liver fibrosis	1/KDR), tNOX, AP-1, etc.	
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, neuromuscolar 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, non-specific 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,	NF-κB targeted inhibitor	Tohoku

		HIV infection, cancers		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).

2.2. Therapeutic usage of NF-κB inhibitors in chronic inflammation

Due to the central role of the IKKγ and Ikkβ molecules within the IKK complex in activating inflammation, the identification of selective IKKβ and IKKγ inhibitors that do not target IKKα or the P100/p52 pathway as therapeutic agents in treating chronic inflammation is of considerable interest. Two specific inhibitors of NF-κB have emerged that appear to be highly therapeutically active in the treatment of several chronic inflammatory diseases, and which provide possible therapeutic approaches to the treatment of PD. The first is a peptide directed against the N-terminal region of NEMO that associates with a hexapeptide sequence within the C-terminus of both IKKα and IKKβ, named the NEMO-binding domain (NBD). This cell permeable peptide spans the NBD and disrupts the association of NEMO with IKKs in vitro and blocks TNFα-induced NF-κB activation in vivo [26]. Notably, the NBD peptide does not affect basal activity of the IKK but only suppresses the induction of activity in response to inflammatory cytokines [27]. Continuous administration of the NBD peptide effectively ameliorates inflammatory responses in animal models without overt signs of toxicity [28]. Additionally, in mouse models of chronic

inflammation, including collagen-induced arthritis (CIA) [29], experimental allergic encephalomyelitis (EAE), Duchenne's muscular dystrophy [30], and inflammatory bowel disease (IBD) [31], *in vivo* treatment with NBD peptides blocked disease activity, inflammatory cytokine expression, and homing of cells to inflammatory sites. Furthermore, mice treated systemically with an NBD peptide for five days after induction of CIA maintained clinical and histological improvement for nearly three weeks following termination of peptide administration [32]. It is important to note that in the therapeutic use of NBD peptide, there was no evidence of undesired off-target effects as the treatment with NBD peptide was shown to be specific for inhibiting NF-κB signaling, exhibiting no inhibitory effects towards JNK or p38 MAPK pathways [33]. The safety profile for NBD is favorable as well. In vivo, systemic delivery of NBD is not associated with any described toxicity in mice or rats, and inhibition of NF-κB has been demonstrated to ameliorate an ever-growing list of inflammatory disease conditions [34]. Therefore, the therapeutic effect of the short-lived NBD peptide may far outlast its pharmacokinetic properties. Thus, selective IKK inhibition by NBD peptides may (i) be an effective therapeutic intervention in chronic inflammatory diseases; (ii) lead to durable alterations in immune responses that correlate with durable clinical efficacy; (iii) minimize potential toxicity concerns associated as basal NF-κB activity remains intact as does the alternative pathway of NF-κB activation necessary for B-cell development and lymphoid organogenesis.

A second approach to the inhibition of inflammation has been to utilize small molecule inhibitors that specifically block the kinase enzymatic activity of IKKβ. One such specific inhibitor, called Compound A, is a small molecule inhibitor of the kinase activity of IKKβ but not IKKα. Compound A, also known as BAY-65-1942 (7-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-5-[(3S)-3-piperidinyl]-1,4-dihydro-2Hpyrido [2,3-d] [1,3]-oxazin-2-one hydrochloride), has been shown to specifically and effectively block the catalytic activity of IKKβ, inhibiting its ability to phosphorylate IκB and activate the cytosolic p50/p65 NF-κB heterodimers [35]. Compound A has been used extensively in vivo, and it has now been found to prevent pulmonary inflammation [36], to attenuate myocardial injury and dysfunction after ischemia-reperfusion injury [37], and to prevent graft versus host disease in murine models of GVHD (Serody et al., personal communication). Other IKKβ inhibitors, including PS-1145 [38] and TPCA-1 [39], have been shown to effectively prevent graft versus host disease in a murine bone marrow transplant model [40], to enhance sensitivity of multiple myeloma cells to chemotherapy by inhibiting the protective effects of NF-κB [41], to inhibit melanoma growth in vivo [42], and to inhibit the growth of colon cancer [43]. These inhibitors work primarily through the inhibition of IKKβ, and their specific suppression of the canonical NF-κB signaling pathway and consequent decrease in serum levels of TNFα and IL-6 are the main features which mediate their inhibitory activity.

Concept and Applications

Nf-κB-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 Nf-κB-targeting agents with high IP value. We apply CDL's proprietary Chemosoft^{TM} software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.

2. Kohonen Self-organizing Maps as a strategic approach to Nf-κB-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 Nf-κB-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 NF-κB-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 signaling pathway.

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 reported NF-kB inhibitors as well as topological pharmacophore are shown in Fig. 3. The modified structural fragments are highlighted in color.

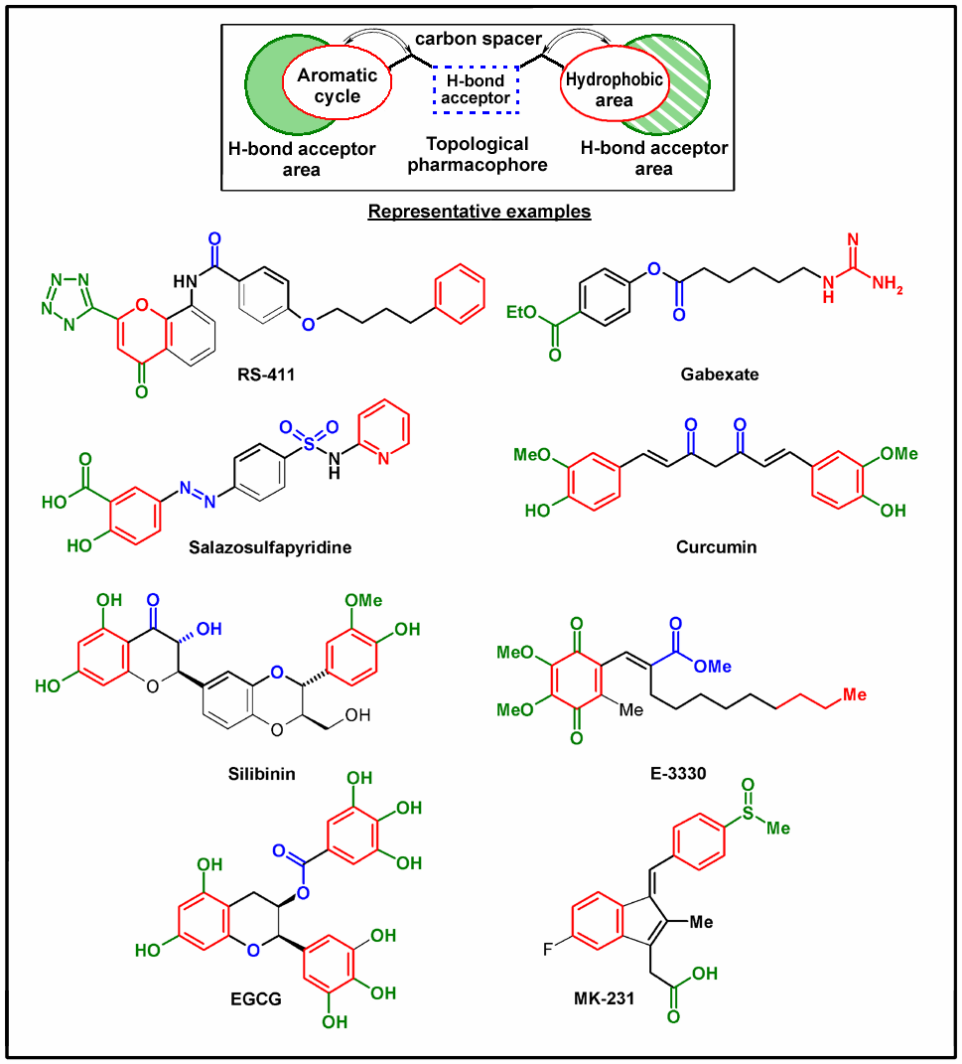


Figure 3. A common topological pharmacophore and bioisosteric transformations of NF-kB inhibitors

As shown in Fig. 3, many of NF-kB inhibitors belonging to different structural classes commonly contain several key structural motifs. 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. 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. 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 500 NF-kB-targeted agents which were shown to have activity against theses targets; representative structures are shown in Fig. 2. 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. 4. 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. 5a-l). In particular, as shown in Fig. 5e, pharmaceutically relevant agents targeted against NF-κB occupy compact/distinct areas within the map constructed. The classification accuracy of

Kohonen modeling was approx. 82% for NF-κB 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.

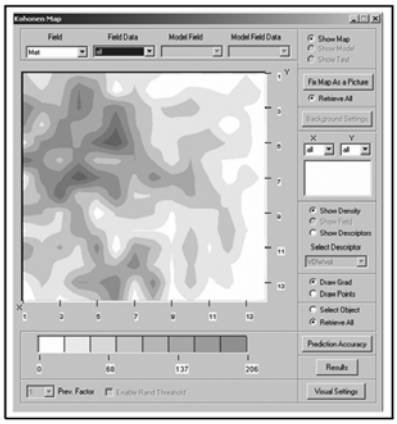


Fig. 4. Property space of 22K pharmaceutical leads and drugs visualized using the Kohonen map. The data have been smoothed

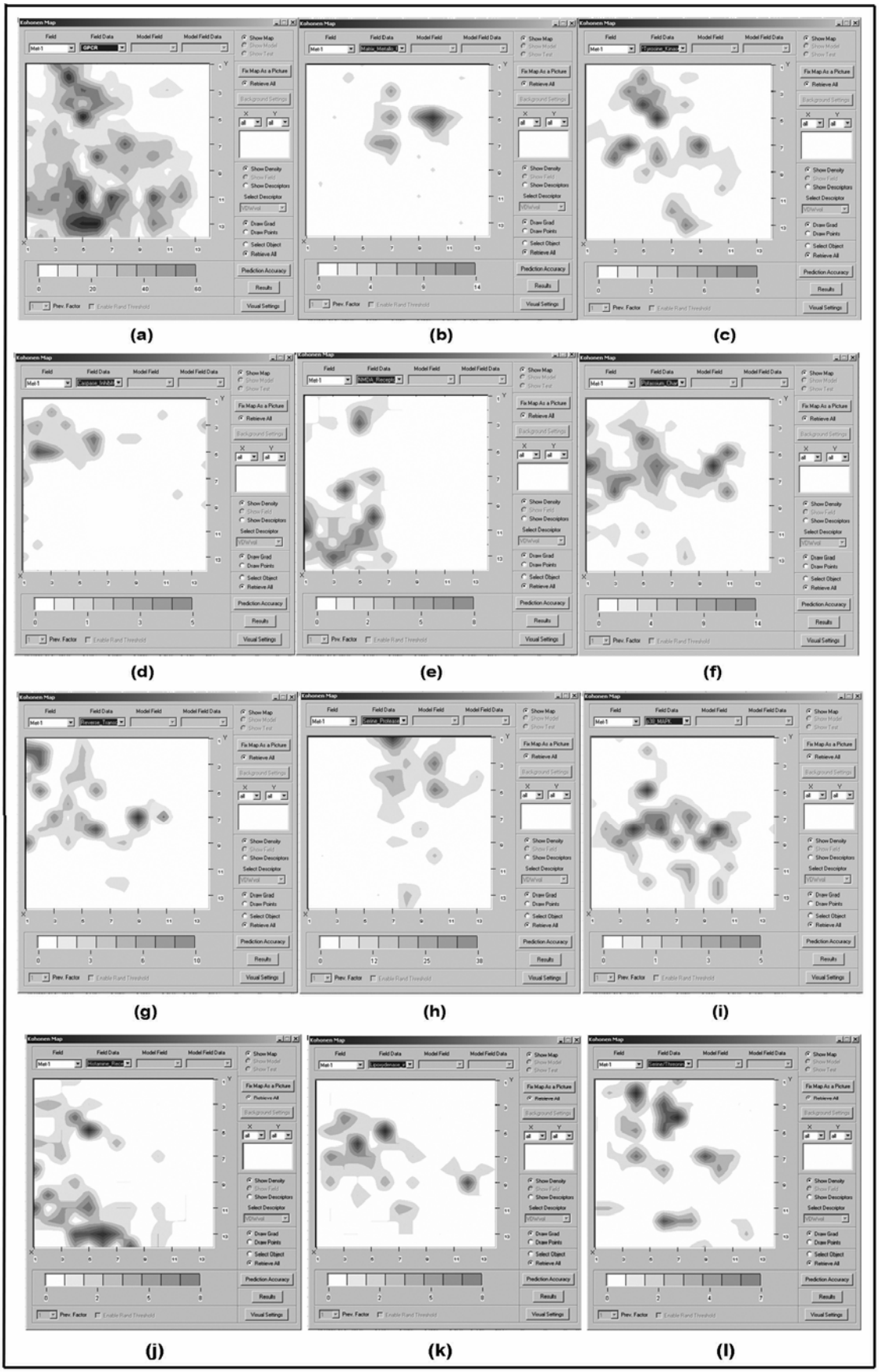


Fig. 5. Distribution of nine large target-specific groups of pharmaceutical agents within the Kohonen map: (a) GPCR agonists/antagonists (5432 compounds); (b) matrix metalloproteinase inhibitors (120 compounds); (c) tyrosine kinase inhibitors (175 compounds); (d) JAK/STAT inhibitors (195 compounds); (e) NF-κB inhibitors (505 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 300K structures of high diversity was exported from ChemDiv database (collection) as an SDF-file with a unique ID-number per each structure. Subsequently, it was imported into the SmartMining software (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. 6,b.

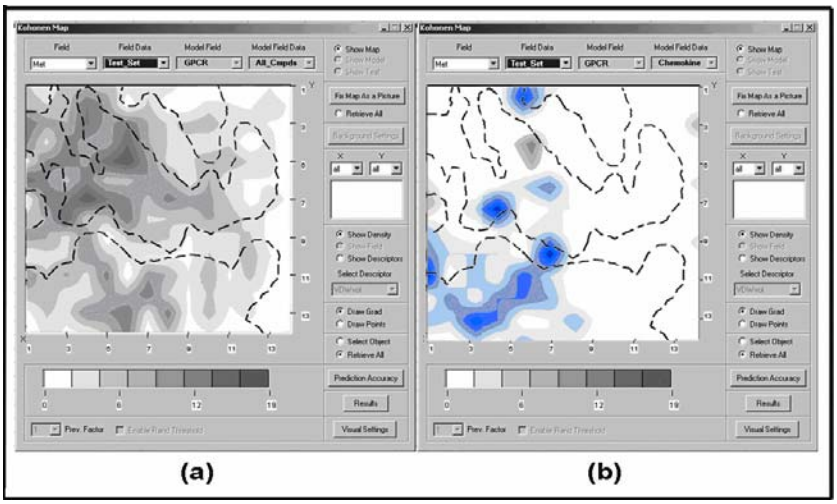


Fig. 6. Distribution of the tested compounds (*dotted line*) within the Kohonen map: (**a**) the overlapping with a whole pharmaceutical area; (**b**) the overlapping with NF-kB area (blue)

The `high-score` compounds which were formally classified by the algorithm as potential NF-kB agents (55K structures) were then exported into an external SDF-file. These 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 structure identification and binding sites determination.[44] 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.[45] Thus, unique 3D-docking models for NF-κB factor 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 15K `high-score` compounds into our NF-κB-library. Representative results of the docking evaluation are presented in Figs. 7-9.

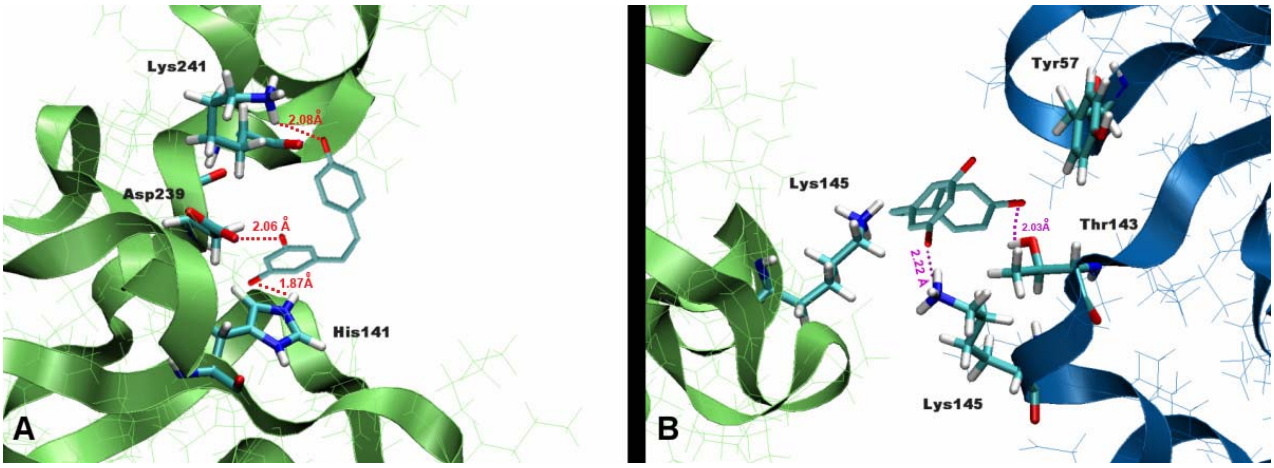


Fig. 7. Binding modes of Resveratrol (**26**) docked into the active site of NF-κB p50: (**A**) monomer and (**B**) homodimer (chain A, shown in green). The residues involved in the interaction with the ligand are shown; the hydrogen bonding and the relative distances are indicated in purple. This model we have used as the `template` binding model

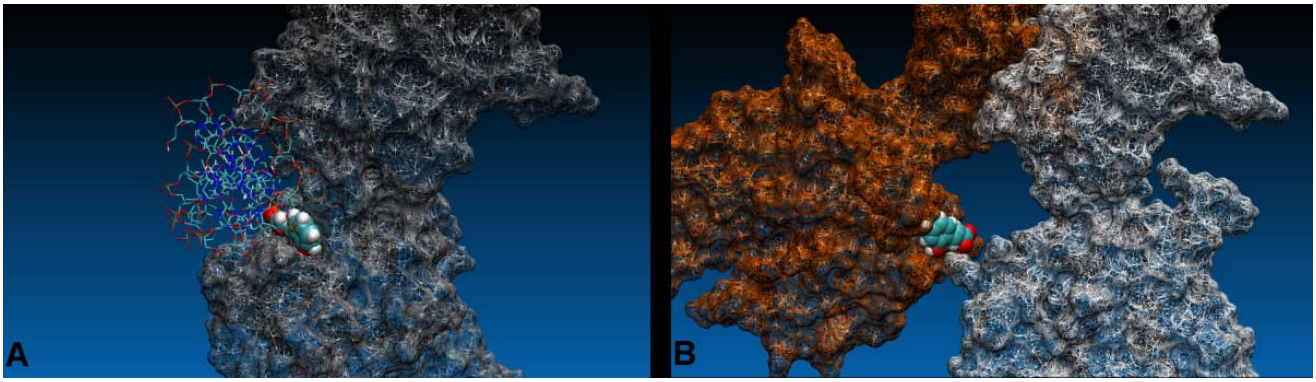
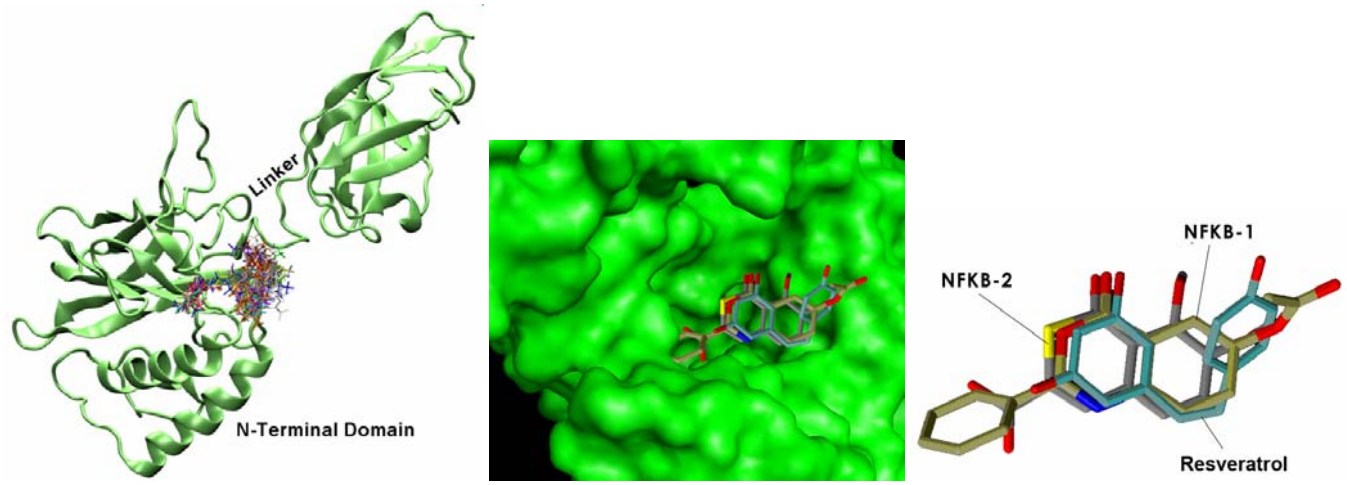


Fig. 8. Poses of docked Resveratrol into the DNA binding region of NF-κB p50. (**A**) monomer and (**B**) homodimer (chain A, shown in green). The inhibitory activity of Resveratrol may be due to its ability to form a stable complex with the active conformation of the dimer and/or blocking the interaction of DNA with the monomer filling the protein binding site. The DNA was obtained from the crystal structure of the homodimer NF-κB. 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. 9. (**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 and two structurally related compounds NFKB-1 and NFKB-2 form Nf-κB-library docked into the DNA binding site of the protein; (**C**) the overlapping between Resvertanol and the tested compounds

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

Synthesis and biological evaluation

(4) Novel Nf-κB-library is synthesized according to the above criteria.

(5) Compounds from Nf-κB-library are planned to be 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 Nf-κB-library consisted of more than 13K small molecule compounds Representative set of Nf-κB-biased compounds is shown in Fig. 10.

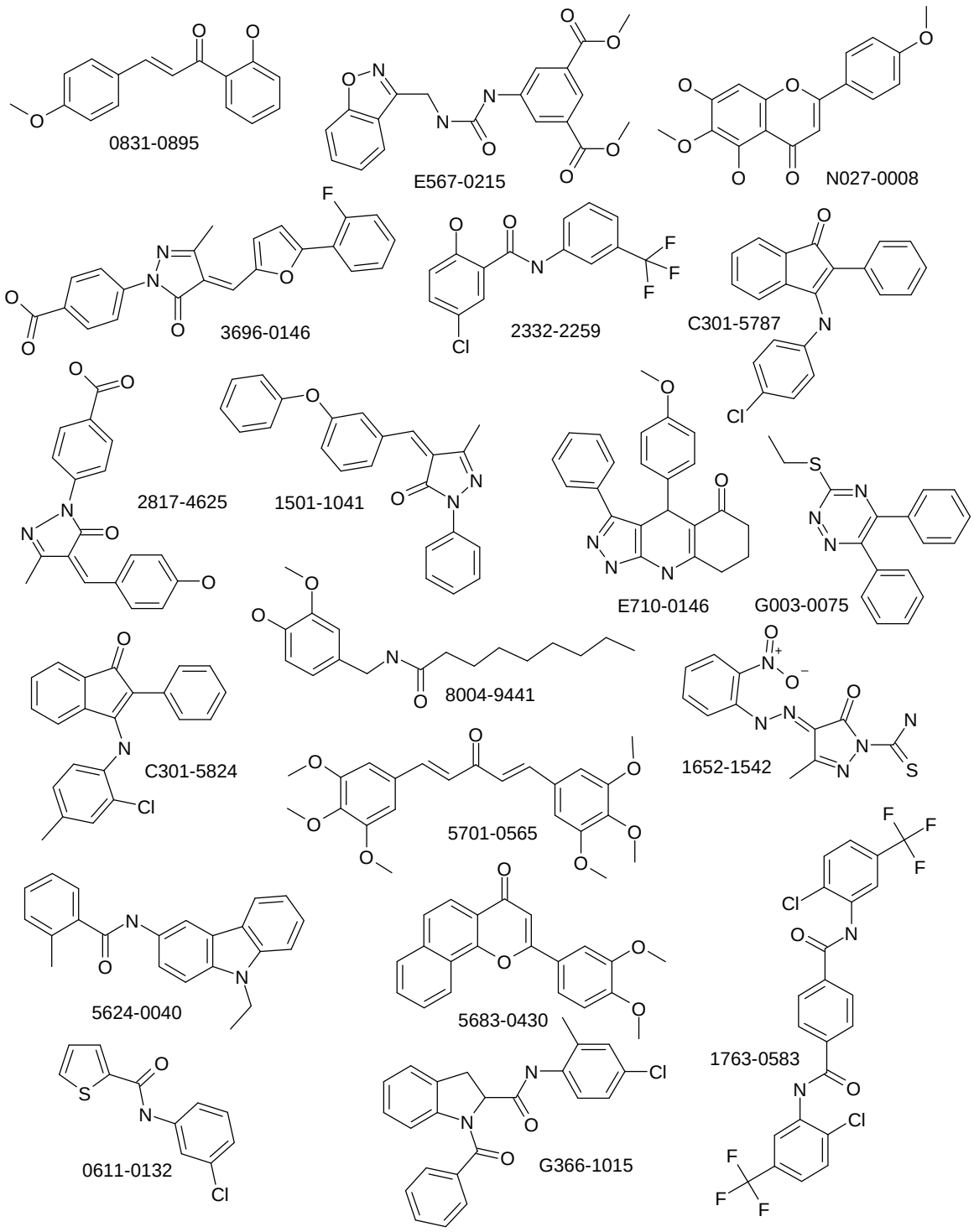


Fig. 10. Representative examples of compounds from NF-κB-targeted library

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 signaling cascade 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 the 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-organazing mapping as well as 3D-molecular docking study. As a result, ChemDiv NF-κB-focused library includes more than 13K `high-score` small molecule compounds specifically targeted against NF-kB factor. 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. 11-15K compounds). As a result, the library is renewed each year, proprietary compounds comprising 65-75% 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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