Chemokine Receptor-Targeted Library

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Chemokine receptors – one of the key targets implicated in inflammation diseases, cancer pathology and viral infections

Chemokines are a family of small proteins inducing directed cell migration via specific chemokine receptors, which play important roles in a variety of biological and pathological processes [1]. Therapeutic strategies based on modulation of chemokine receptor pathways were reported to be promising clinical strategies in the treatment of inflammatory diseases, such as multiple sclerosis and atherosclerosis [2], psoriasis, inflammatory skin diseases and atopic dermatitis [3], as well as viral infections, including HIV [4]. Chemokine involvement is not limited to immunity and inflammation. Recent studies have clearly demonstrated that chemokines and chemokine receptors are produced by many different cell types, including tumor cells [1,5]. Since their discovery, the field of chemokine acting has been intimately connected to cancer biology. Overexpression of many chemokine and chemokine receptors in tumor cells suggests that they are crucial regulators of the levels of tumor infiltrating leukocytes implicated in the tumorigenesis of multiple human cancers. For example, CCL5 is highly produced by breast carcinoma, melanoma and ovarian cancer [6]. Melanoma is probably the most studied cancer type in which CCL (CCL2,5,10,21,27) and CXC chemokines in particular CXCL1 and related molecules (CXCL2,3,8 or IL-8) have been demonstrated to play a critical role in tumor progression [7]. Chemokine CXCL1 and its specific receptor CXCR1 were initially identified from supernatants of melanoma cell lines and characterized as autocrine growth stimulators [8]. Blocking of CXCL1-3, or their receptor with specific antibodies inhibited the growth and colony formation of melanoma cells [9]. Several other studies have proposed a similar role for CXCL8 related chemokines as well as CXCR2-4 chemokine receptors in head and neck [10] pancreas [11] and Non-Small-Cell Lung Cancer (NSCLC) [12]. Many published reviews address the significant role of chemokine receptors in regulating angiogenesis [13], metastasis, adhesion, invasion, growth and cancer progression [14]. Recent pre-clinical and clinical studies have reported the promising anti-cancer activity of chemokine receptor antagonists, in several cancer models. Among a heap of chemokine receptors CXCR1/2/4, CX3CR and CCR1/2/3/5 are the most actively studied signal transmitters which possess multiple critical functions in normal and pathologic physiology. In particular, the CXCL12/CXCR4 signaling system was found to be deeply involved in tumor cells migration and metastases formation in mammals. Many reports have shown that CC chemokines CCL3-5 strongly inhibit HIV-1 production in newly infected peripheral blood mononuclear cells. To enter target cells, HIV type 1

(HIV-1) requires two distinct recognition elements: CD4 and either CXCR4 or CCR5, suggesting a mechanism for the inhibition of HIV infection by some chemokines [15].

Ligand-receptor relationships within the chemokine superfamily are extremely complex. The receptors have been operationally subdivided according to the complexity of their relationships to ligands into various groups. Chemokine receptors, like all members of the GPCR superfamily, mediate signal transduction through specific G-proteins. Although chemokine receptors are morphologically similar to many other 7-TMS receptors, they have several unique structural signatures such as the amino acid sequence DRYLAIV in the second intracellular loop domain [16]. Chemokine signaling has been studied most comprehensively for CCR2, CCR5, CXCR1 and CXCR4 in neutrophils, macrophages, T-cells [17], and more recently also in dendritic cells [18]. It should also be noted, that in many cases multiple receptors can be activated by a single chemokine, and it is anticipated that multiple chemokine receptor antagonists may be more effective than compounds selectively acting against a single receptor. Finally, it was suggested that combinations of CCR2-CCR3, CCR1-CCR3 and CCR3-CCR5 were the most feasible.

Concept and Applications

CR-targeted library design at CDL involves:

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

1. Unique morphing and funneling procedures in designing novel potential CR ligands with high IP value. We apply CDL's proprietary Chemosoft^{TM} software and commercially available solutions from Accelrys, MOE, Daylight and other platforms.

2. Neural Network tools for target-library profiling, in particular Self-organizing Kohonen Maps, performed in SmartMining Software.

3. A molecular docking approach to focused library design.

5. Chemogenomics approach to target 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 CR-focused libraries:

Virtual screening

(1) The small-molecular ligands for all CR classes are compiled into a unique knowledge base (reference ligand space) and annotated according to the particular receptor subtype. The knowledge base is analyzed for the pharmacophore hypotheses (Fig. 1) and particular bioisosteric rules (Fig. 2) used in the subsequent morphing procedures.

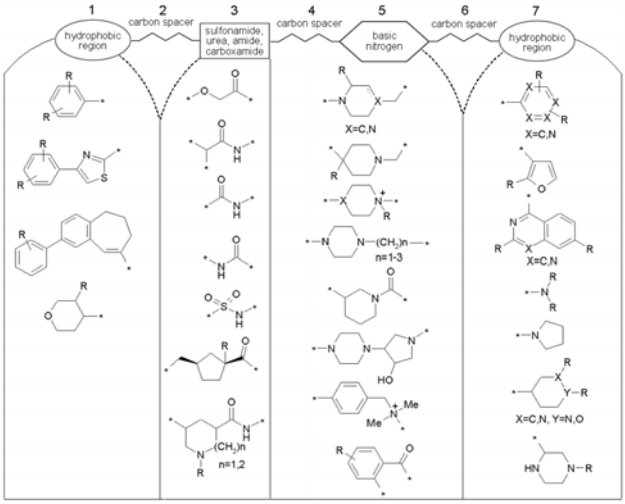
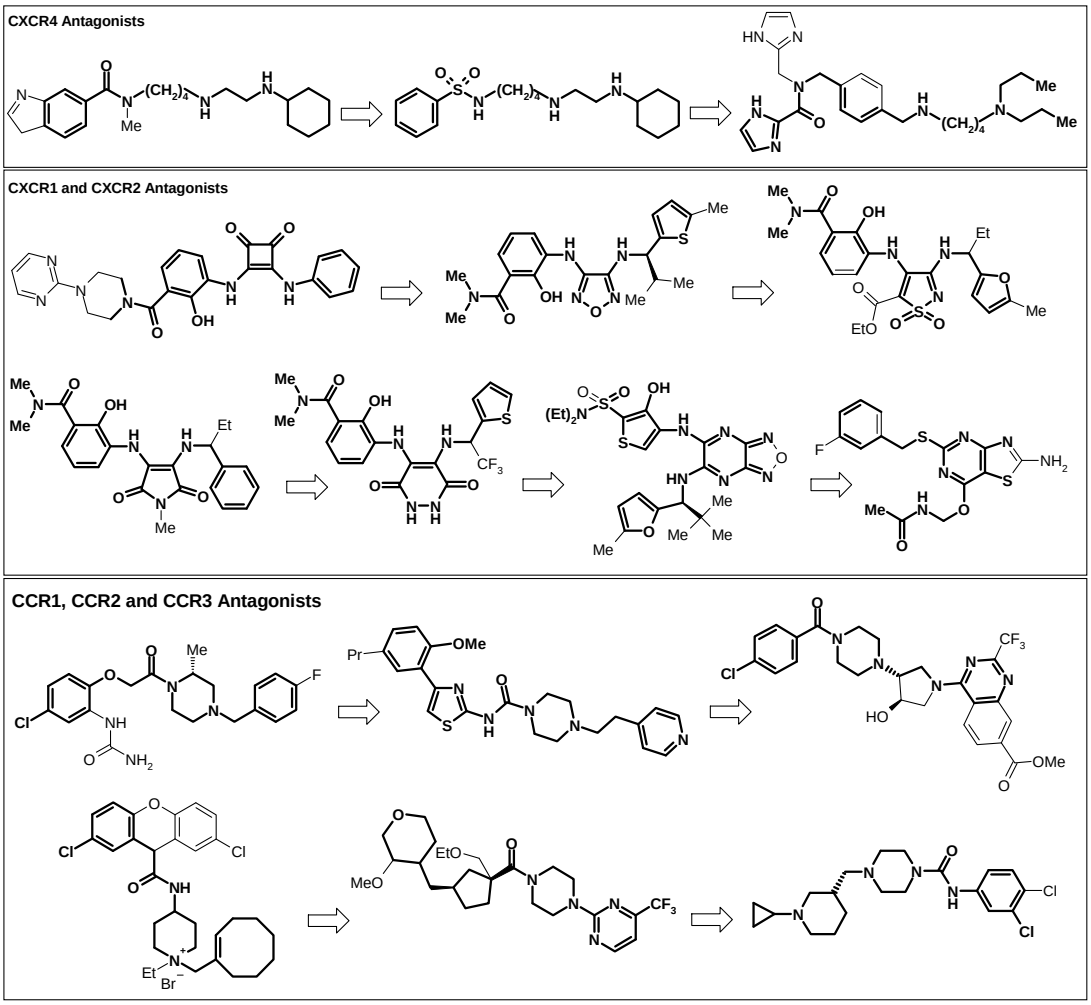
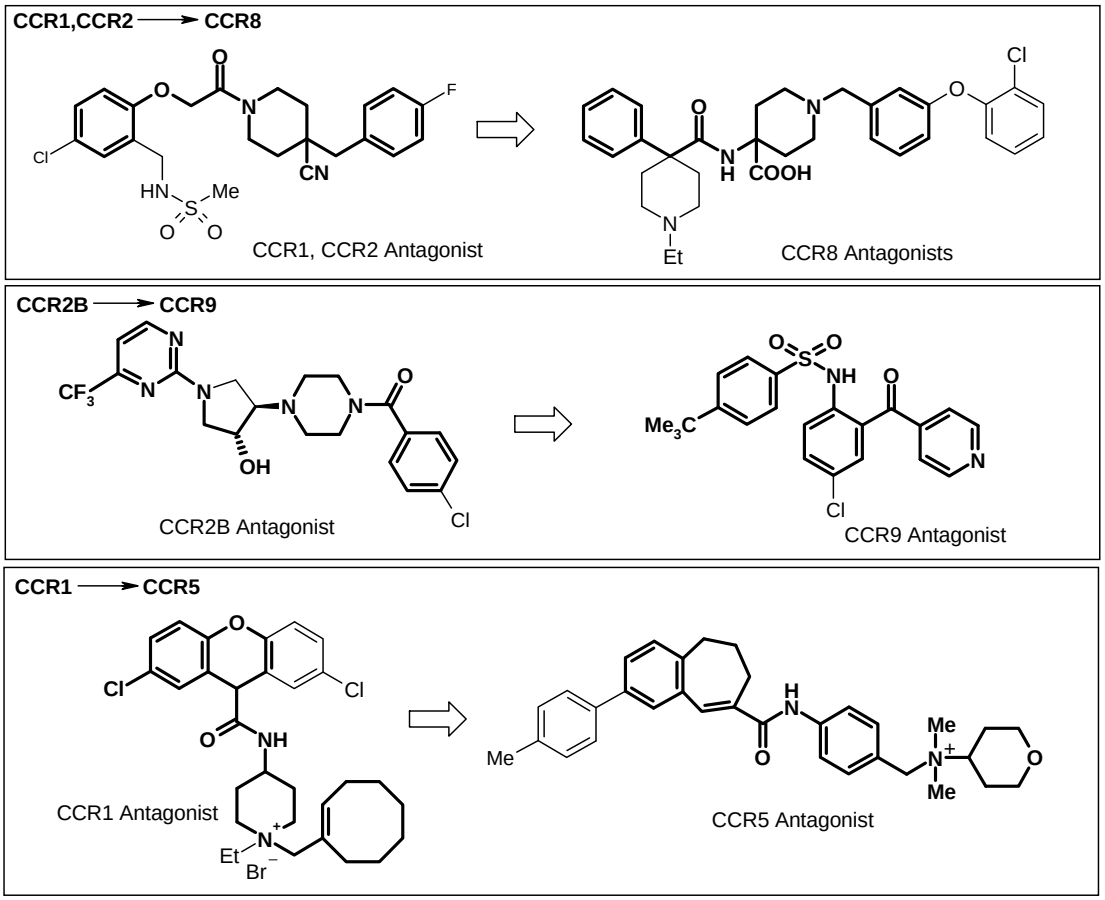


Fig. 1. Putative pharmacophore model for CCR-chemokine receptors antagonists



A



B

Fig. 2. (**A**) Typical examples of bioisosteric modifications for representative small molecule antagonists acting within individual chemokine receptor classes; (**B**) Bioisosteric and topological transformations for antagonists acting against different chemokine receptors

The reference ligand space is used as a source for designing novel chemotypes via a variety of structure morphing procedures: bioisosteric, pharmacophore, 2D and 3D similarity.

(2) One of the difficult challenges in data analysis is to be able to represent whatever complexities might be intrinsic to the data in a simple and intuitive form. In order to minimize the complexity and reduce number of individual plots needed to visualize this sort of data, one must attempt to reduce the dimensionality of the representation. Several different techniques were proposed to achieve dimensionality reduction, while preserving the topology of the original space. That is, points near each other in the high-dimensional space are also near each other in the low-dimensional space. Among these algorithms Kohonen-based SOM's and Sammon maps are the most widely used techniques for dimensionality reduction with different conceptual basis.

In particular, Self-organizing Kohonen maps [19**]** belong to a class of neural networks known as competitive learning or self-organizing networks which in turn are based on unsupervised learning rule (*see notes*). 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 [for review, see: 20(a-f)].

We have used this approach for compound selection and focused-library profiling. Thus, the collected training data set consisted of known 16540 drug-compounds, including 1212 CR-antagonists, was then filtered and preprocessed. Various molecular descriptors were calculated for Kohonen modeling using SmartMining software. 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. The whole Self-organizing Kohonen Map of 16 thousand pharamaceutical leads and drugs generated as a result of the unsupervised learning procedure is depicted in Fig. 3.

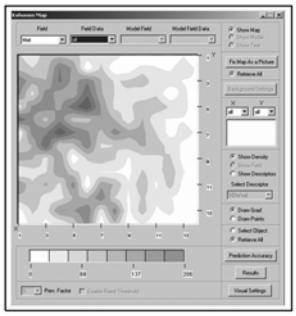


Fig. 3. Property space of 16000 pharmaceutical leads and drugs visualized using the Kohonen map. The data have been smoothed

Distribution of representative target-specific groups of GPCR-ligands within the Kohonen map demonstrates that most of these groups have distinct locations in specific regions of the map (Fig. 4).

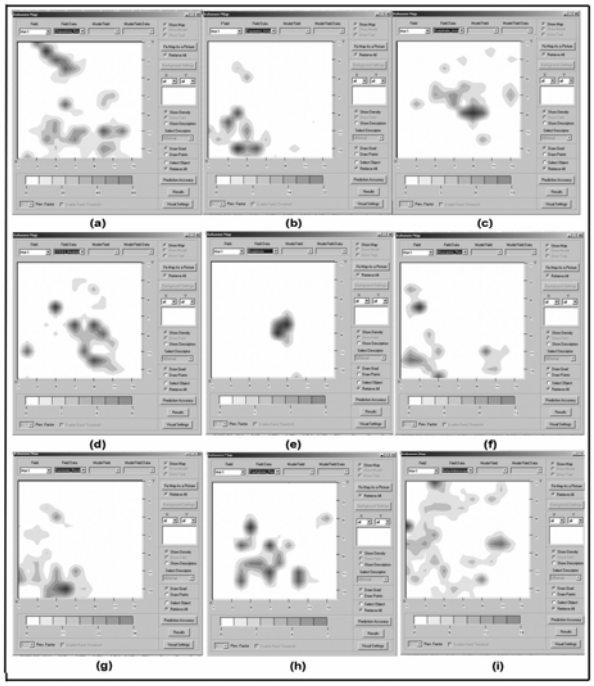


Fig. 4. Distribution of several GPCR-specific groups of pharmaceutical agents on the Kohonen map: (a) chemokine receptors agonists/antagonists (1212 compounds); (b) dopamine D1-D4 agonists/antagonists (320 compounds); (c) endothelin ETA/ETB

antagonists (121 compounds); (d) CCKA/B agonists/antagonists (77 compounds); (e) bradykinin agonists/antagonists (12 compounds); (f) muscarinic M1 agonists (121 compounds); (g) serotonin receptors agonists/antagonists (477 compounds); (h) tachykinin NK1/NK2 antagonists (103 compounds); (i) β-adrenoceptor agonists/antagonists (556 compounds)

As shown in Fig. 4, compounds targeted specifically on different GPCR subclasses including α/β-adrenoceptors, dopamine D1-D4 receptors, tachykinin NK1/NK2, serotonin and chemokine receptors can be successfully separated within the map constructed. The distribution of six chemokine-specific groups of pharmaceutical agents within the Kohonen map is shown in Fig. 5.

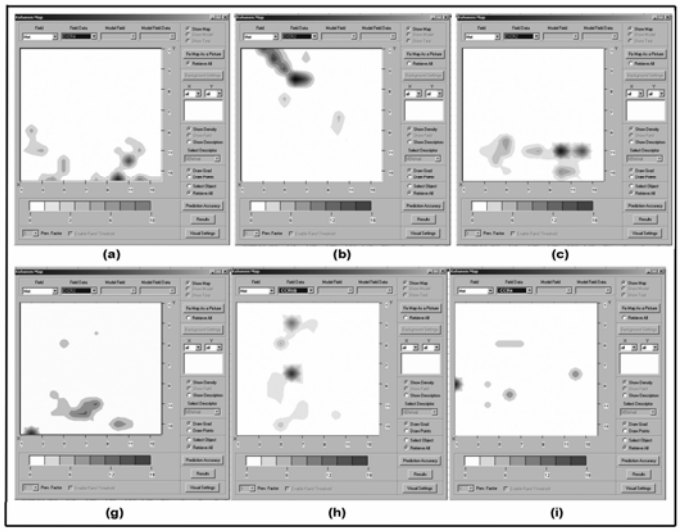


Fig. 5. Distribution of CR-targeted pharmaceutical agents within the Kohonen map: (a) CXCR4 antagonists (80 compounds); (b) CXCR1/2 antagonists (442 compounds); (c) CCR5 antagonists (238 compounds); (d) CCR3 antagonists (182 compounds); (e) CCR1/2 antagonists (180 compounds); (f) CCR4 antagonists (13 compounds)

We have used this model for rational compound selection. Thus, the generated virtual library had been tested and structures were classified in different groups in accordance to the predicted activity, in particular against CXCR4 repeptor (Fig. 6).

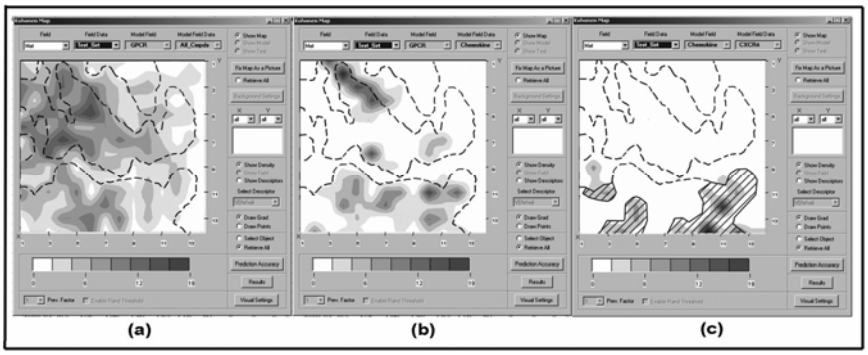
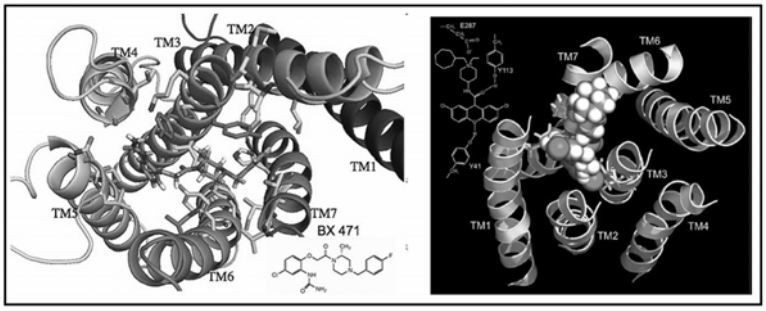


Fig. 6. Distribution of the tested compounds (dotted line) within the Kohonen map: (a) the overlapping with GPCR agonists/antagonists area; (b) the overlapping with chemokine receptor antagonists areas; (c) the selection of compounds which can be regarded as potential agents acting against CXCR4 chemokine receptor (shaded area)

(3) A major challenge in the application of structure-based drug design methods to chemokine receptors is the paucity of structural information. Therefore, several actual studies were targeted for the chemokine

receptors structure identification and binding sites determination. For instance, using the MembStruk computational method, three-dimensional structure of the human CCR1 chemokine receptor and binding site of the small molecule selective CCR1 antagonists BX-471 and UCB-35625 were recently reported (Fig. 7) [21].



A **B**

Fig. 7. BX-471 (**A**) and UCB-35625 (**B**) bounded to human CCR1 chemokine receptor.

A classical target-based design using molecular docking approach was recently carried out to provide a basis for the development of novel small molecule antagonists of CCR2 [23], CCR5 [24]. We have effectively used the data and 3D-models listed above for generation of the CR-focused library and further structure optimization.

(4) During the past decade, the main paradigm in medicinal chemistry has been turning gradually from traditional receptor-specific studies and biological assays to a novel cross-receptor vision. Currently, such approach becomes increasingly applied within the whole pharmaceutical research to enhance the efficiency of modern drug discovery. Among these methods, chemogenomics comprises a special discipline targeted particularly at systematically studying the biological effect of a vast number of small-molecular compounds on a wide spectrum of principal biological targets. This strategy is fundamentally based entirely on the paradigm originally introduced by Klabunde **[**25**]** who has formulated the basic principle - '*similar receptors bind similar ligands*'. So, we have used this strategy for the CR-target library design.

The chemokine superfamily includes a large number of ligands that bind to a smaller number of receptors [1,26], at that multiple chemokine ligands can bind to the same receptor and vice versa. Whereas the perceived complexity and promiscuity of receptor binding introduce an additional challenge in understanding the common mechanism of chemokine ligands binding, with respect to chemogenomics they provide a valuable starting point to investigate key interrelationships across chemokine receptor subfamily. Currently, there are more than 20 functionally signaling chemokine receptors and more than 45 corresponding chemokine ligands in humans [1]. The chemokine ligands and receptors have been divided into several major groups based on their expression patterns and functions. Their genomic organization also provides an alternative CRs-classification. This is apparent from the phylogenetic tree presented in Fig. 8.

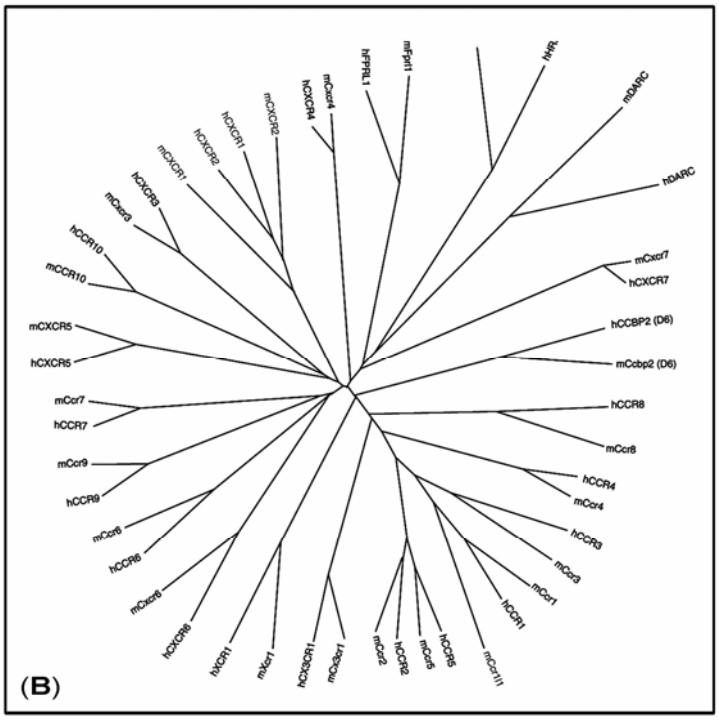


Fig. 8. Sequence relationship analysis of the human (h) and mouse (m) chemokine receptors.

As shown in phylogenetic dendogram, CXCR4 is located closely to CXCR1, CXCR2 as well as CXCR3. This means primarily that these receptors possess a similar genotype and based on this observation they can be logically grouped into the common CXCR family differed genetically from CCR subclass but not significantly. Prima facie, it seems to be perfectly reasonable to investigate small molecule space around whole CXCR subclass however there are different similarity criteria which should be thoroughly considered. In addition, a binding site composition and corresponding space cavity jointly play a key role in the ligand binding process. Furthermore, the majority of ligand-receptor complexes are not static structures, they can change dynamically upon ligand binding. Thus, enforced conformational changes across the active binding site can also be achieved by ligand partial binding followed by internal cavity formation fitted appropriately for deep embedding. It is particularly interesting that there are several scientific reports highlighted the partial sequence homology (25-30%), while there is a high binding sites similarity between CCR5 and CXCR4 [27]. In addition, a high degree of similarity was also determined for CCR5 and CCR3 [28]. Therefore, from the chemogenomics point of view, it is of practical relevance to test the agents acting against CXCR4 also on activity towards CXCR1-3 and CCR3/5. Thus, compounds are profiled against a set of receptors and not tested against single targets. We have effectively used this modern approach and related theoretical basis for the analysis of the selected structures and their target-specific profile obtained from the biological screening outcome.

(5) A series of consecutive funneling procedures are applied to enhance the target-specific relevance of novel compounds. During this step, we address the compound's lead-likeness (enforcing partial Rule of 3 compliance18), the availability of unique R-groups, the pre-synthetic analysis of privileged templates, the IP potential, the feasibility of high-throughput chemistry. We also consider the ADME/Tox issues (such as

HIA and BBB-permeability, plasma protein binding, cytochrome P450 substrate and inhibition potential) and key physico-chemical properties (such as DMSO and water solubility, stability and ionization potential, etc.). The funneling procedures are carried out by CR-specific neural networks, fragment and property-based models. Diversity of the final selection is optimized using proprietary algorithms.

Synthesis and biological evaluation

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

(5) The subsets of CR 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 CR-focused library consisted of more than 8000 small molecule compounds targeted particularly against CXCR1/2 receptors. Representative set of CR-biased compounds is shown below.

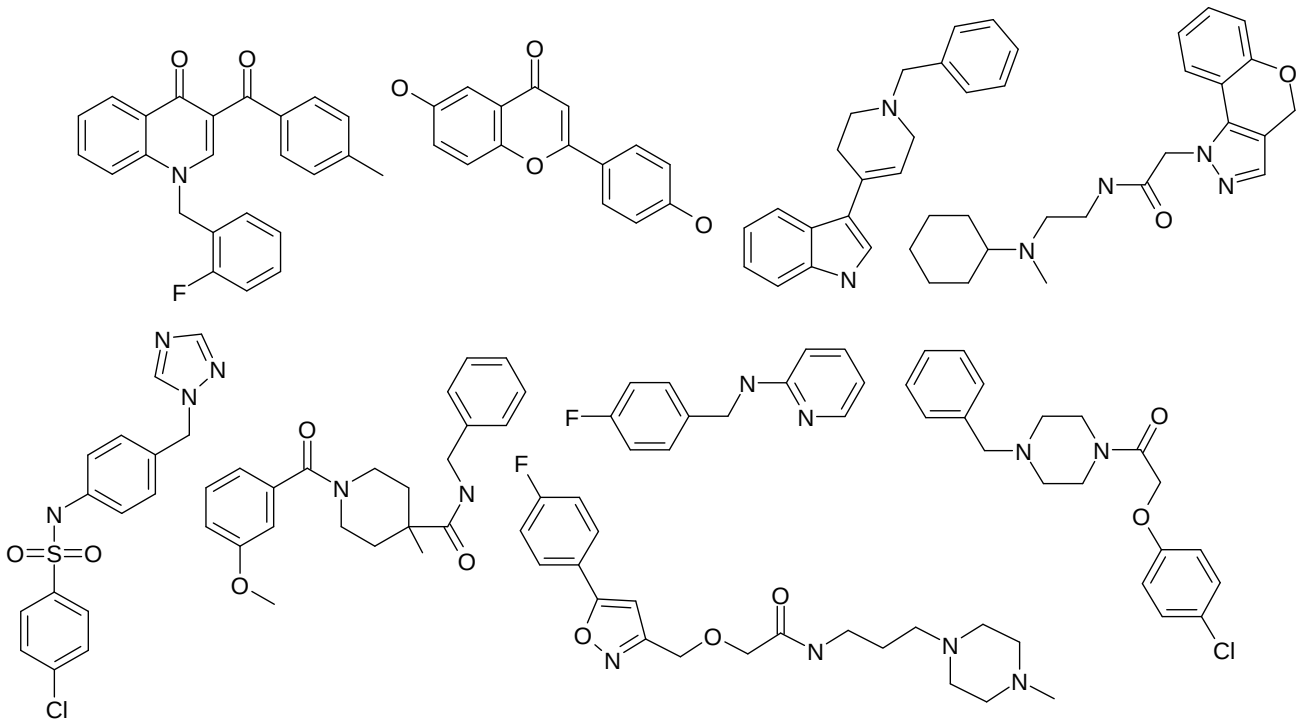


Fig. 9. Examples of compounds from the CR-targeted library

We provide rapid and efficient tools for follow-up chemistry on discovered hits, including single isomer chemistry, stereoselective synthesis and racemic mixture separation. Targeted 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. 8-10K 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.

References

1. (a) Balakin, K. V., Ivanenkov, Y. A., Tkachenko, S. E., Kiselyov, A. S., and Ivachtchenko, A. V. (2008) Regulators of chemokine receptor activity as promising anticancer therapeutics *Curr Cancer Drug Targets* **8,** 299-340; (b) Lavrovsky, Yan; Ivanenkov, Yan A.; Balakin, Konstantin V.; Medvedeva, Darya A.; Ivachtchenko, Alexandre V. (2008) CXCR4 Receptor as a Promising Target for Oncolytic Drugs *Mini Reviews in Medicinal Chemistry* **8**, 1075-1087.

2. (a) Sorensen, P. S. Biological Markers in Body Fluids for Activity and Progression in Multiple Sclerosis. *Mult. Scler*. **1999**, *5*, 287–290); (b) Israel, F.; Charo, M. D.; Richard, M.; Ransohoff, M. D. The Many Roles of Chemokines and Chemokine Receptors in Inflammation. *The new England journal of medicine* **2006**, *354*, 610-21.

3. Vestergaard, C.; Deleuran, M.; Gesser, B.; Gronhoj, L. C. Expression of The T-Helper 2-Specific Chemokine Receptor CCR4 on CCR10-Positive Lymphocytes in Atopic Dermatitis Skin but Not in Psoriasis Skin. *Br. J. Dermatol*. **2003**, *149*, 457–463.

4. Hoffman, T. L.; Doms, R. W. Chemokines and Coreceptors in HIV/SIV-host Interactions. *AIDS* **1998**, *12 Suppl A*, S17-26, 5. Horuk, R. Chemokines. *Scientific World Journal* **2007**, *19*, 224-32.

5. Choi, C.; Xu, X.; Oh, J. W.; Lee, S. J.; Gillespie, G. Y.; Park, H.; Jo, H.; Benveniste, E. N. Fasinduced Expression of Chemokines in Human Glioma Cells: Involvement of Extracellular Signal-regulated Kinase 1/2 and p38 Mitogen-activated Protein Kinase. *Cancer Res*. **2001**, *61*, 3084–3091.

6. Mrowietz, U.; Schwenk, U.; Maune, S.; Bartels, J.; Küpper, M.; Fichtner, I.; Schröder, J. M.; Schadendorf, D. The Chemokine RANTES is Secreted by Human Melanoma Cells and is Associated with Enhanced Tumour Formation in Nude Mice. *Br. J. Cancer* **1999**, *79*, 1025-31.

7. Haghnegahdar, H.; Du, J.; Wang, D.; Strieter, R. M.; Burdick, M. D.; Nanney, L. B.; Cardwell, N.; Luan, J.; Shattuck-Brandt, R.; Richmond, A. The Tumorigenic and Angiogenic Effects of MGSA/GRO Proteins in Melanoma. *J. Leukoc. Biol*. **2000**, *67*, 53-62.

8. Bordoni, R.; Fine, R.; Murray, D.; Richmond, A. Characterization of The Role of Melanoma Growth Stimulatory Activity (MGSA) in The Growth of Normal Melanocytes, Nevocytes, and Malignant Melanocytes. *J. Cell Biochem*. **1990**, *44*, 207-19.

9. Owen, J. D.; Strieter, R.; Burdick, M.; Haghnegahdar, H.; Nanney, L.; Shattuck-Brandt, R.; Richmond, A. Enhanced Tumor-Forming Capacity for Immortalized Melanocytes Expressing Melanoma Growth Stimulatory Activity/Growth-Regulated Cytokine Beta and Gamma Proteins. *Int. J. Cancer* **1997**, *73*, 94-103.

10. Richards, B. L.; Eisma, R. J.; Spiro, J. D.; Lindquist, R. L.; Kreutzer, D. L. Coexpression of Interleukin-8 Receptors in Head and Neck Squamous Cell Carcinoma. *Am. J. Surg*. **1997**, *174*, 507-12.

11. Takamori, H.; Oades, Z. G.; Hoch, O. C.; Burger, M.; Schraufstatter, I. U. Autocrine Growth Effect of IL-8 and Groalpha on a Human Pancreatic Cancer Cell Line, Capan-1. *Pancreas* **2000**, *21*, 52-6.

12. Olbina, G.; Cieslak, D.; Ruzdijic, S.; Esler, C.; An, Z.; Wang, X.; Hoffman, R.; Seifert, W.; Pietrzkowski, Z. Reversible Inhibition of IL-8 Receptor B Mrna Expression and Proliferation in Non-Small Cell Lung Cancer by Antisense Oligonucleotides. *Anticancer Res*. **1996**, *16*, 3525-30.

13. Koch, A. E.; Polverini, P. J.; Kunkel, S. L.; et al. Inteleukin-8 as a Macrophage-derived Mediator of Angiogenesis. *Science* **1992**, *258*, 1798-1801.

14. Murphy, P. M. Chemokines and The Molecular Basis of Cancer Metastasis. *N. Engl. J. Med*. **2001**, *345*, 833-835.

15. Romagnani, P. L.; Lasagni, L.; Annunziato, F.; Serio, M.; Romagnani, S. CXC Chemokines: The Regulatory Link Between Inflammation and Angiogenesis. *Trends. Immunol*. **2004**, *25*, 201-9.

16. Murphy, P. M. The Molecular Biology of Leukocyte Chemoattractant Receptors. *Annu. Rev. Immunol*. **1994**, *12*, 593-633.

17. Proudfoot, A. E. I.; Power, C. A.; Rommel, C.; Wells, T. N. Strategies for Chemokine Antagonists as Therapeutics. *Semin. Immunol*. **2003**, *15*, 57–65.

18. Mellado, M.; Rodr´ıguez-Frade, J. M.; Vila-Coro, A. J.; Fern ´ andez, S.; Martin de Ana, A.; Jones, D. R.; Tor ´ an, J. L.; Mart´ınez-A, C. Chemokine Receptor Homo- or Heterodimerization Activates Distinct Signaling Pathways. *EMBO J*. **2001**, *20*, 2497–2507.

19. Kohonen, T. (1990) The self-organizing map. *Proceedings of the IEEE* **78,** 1464-80.

20 (a) Bauknecht, H., Zell, A., Bayer, H., Levi, P., Wagener, M., Sadowski, J., and Gasteiger, J. (1996) Locating biologically active compounds in medium-sized heterogeneous datasets by topological autocorrelation vectors: dopamine and benzodiazepine agonists *J Chem Inf Comp Sci* **36,** 1205-13.15; (b) Anzali, S., Barnickel, G., Krug, M., Sadowski, J., Wagener, M., Gasteiger, J., and Polanski, J. (1996) The comparison of geometric and electronic properties of molecular surfaces by neural networks: application to the analysis of corticosteroid binding globulin activity of steroids *J Comp-Aid Mol Des* **10,** 521-34: (c) Brűstle, M., Beck, B., Schindler, T., King, W., Mitchell, T., and Clark, T. (2002) Descriptors, physical properties, and drug-likeness *J Med Chem* **45,** 3345-55; (d) Rabow, A. A., Shoemaker, R. H., Sausville, E. A., and Covell, D. G. (2002) Mining the National Cancer Institute's tumor-screening database: identification of compounds with similar cellular activities *J Med Chem* **45,** 818-40; (e) Korolev, D., Balakin, K. V., Nikolsky, Y., Kirillov, E., Ivanenkov, Y. A., Savchuk, N. P., Ivashchenko, A. A., and Nikolskaya, T. (2003) Modeling of human cytochrome P450-mediated drug metabolism using unsupervised machine learning approach *J Med Chem* **46,** 3631-43; (f) Savchuk, N. P. (2003) In silico ADME-Tox as part of an optimization strategy *Curr Drug Disc* **4,** 17-22.

21. Vaidehi, N.; Schlyer, S.; Trabanino, R. J.; Floriano, W. B.; Abrol, R.; Sharma, S.; Kochanny, M.; Koovakat, S.; Dunning, L.; Liang, M.; Fox, J. M.; de Mendonca, F. L.; Pease, J. E.; Goddard, W. A. 3rd.; Horuk, R. Predictions of CCR1 Chemokine Receptor Structure and BX 471 Antagonist Binding Followed by Experimental Validation. *J. Biol. Chem*. **2006**, *281*, 27613-20.

22. de Mendonca, F. L.; da Fonseca, P. C.; Phillips, R. M.; Saldanha, J. W.; Williams, T. J.; Pease, J. E. Site-Directed Mutagenesis of CC Chemokine Receptor 1 Reveals The Mechanism of Action of UCB 35625, a Small Molecule Chemokine Receptor Antagonist. *J. Biol. Chem*. **2005**, *280*, 4808-16.

23. Berkhout, T. A.; Blaney, F. E.; Bridges, A. M.; Cooper, D. G.; Forbes, I. T.; Gribble, A. D.; Groot, P. H.; Hardy, A.; Ife, R. J.; Kaur, R.; Moores, K. E.; Shillito, H.; Willetts, J.; Witherington, J. CCR2: Characterization of The Antagonist Binding Site from a Combined Receptor Modeling/Mutagenesis Approach. *J. Med. Chem*. **2003**, *46*, 4070-86.

24. (a) Fano, A.; Ritchie, D. W.; Carrieri, A. Modeling The Structural Basis of Human CCR5 Chemokine Receptor Function: from Homology Model Building and Molecular Dynamics Validation to Agonist and Antagonist Docking. *J. Chem. Inf. Model*. **2006**, *46*, 1223-35; (b) Paterlini, M. G. Structure Modeling of The Chemokine Receptor CCR5: Implications for Ligand Binding and Selectivity. *Biophys J.* **2002**, *83*, 3012-31; (c) Kellenberger, E.; Springael, J. Y.; Parmentier, M.; Hachet-Haas, M.; Galzi, J. L.; Rognan, D. Identification of Nonpeptide CCR5 Receptor Agonists by Structure-based Virtual Screening. *J. Med. Chem*. **2007**, *50*, 1294-303.

25. Klabunde, T. (2006) Chemogenomic approaches to ligand design. *In* Ligand design for G-protein-coupled receptors, Rognan, D., (Ed). Wiley-VCH, Weinheim.

26 (a) Zlotnik, A., and Yoshie, O. (2000) Chemokines: a new classification system and their role in immunity *Immunity* **12,** 121-7; (b) Yoshie, O., Imai, T., and Nomiyama, H. (2001) Chemokines in immunity *Adv Immunol* **78,** 57-110.

27. (a) Pérez-Nueno, V. I., Ritchie, D. W., Rabal, O., Pascual, R., Borrell, J. I., and Teixidó, J. (2008) Comparison of ligand-based and receptor-based virtual screening of HIV entry inhibitors for the CXCR4 and CCR5 receptors using 3D ligand shape matching and ligand-receptor docking *J Chem Inf Model* **48,** 509-33; (b) Spencer, E. H. (2005) Development of a Structure Prediction Method for G-Protein Coupled Receptors, Thesis, California Institute of Technology.

28. Efremov, R., Truong, M. J., Darcissac, E. C., Zeng, J., Grau, O., Vergoten, G., Debard, C., Capron, A., and Bahr, G. M. (2001) Human chemokine receptors CCR5, CCR3 and CCR2B share common polarity motif in the first extracellular loop with other human G-protein coupled receptors *European Journal of Biochemistry* **263,** 746-56.