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The use of ligand-based de novo design for scaffold hopping and sidechain optimization: Two case studies

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Abstract—This paper describes the application of de novo design utilizing exclusively ligand information. In the current approach, ligand design criteria, including pharmacophores, similarity and desired properties are applied as part of a fitness function driving the design process, instead of using them as filters after the process. This allows relevant parts of chemical space to be explored more efficiently. Two case studies of successful ligand design are also presented, one aimed at scaffold hopping, the other for exploring substitution patterns around a novel scaffold.

1. Introduction

The ultimate success of computer-aided drug discovery would be the de novo design of new drugs using in silico models, even though this is currently too ambitious an aim. Nonetheless, during the drug discovery process it is often necessary to introduce significant changes in the chemical structure of lead compounds for reasons such as selectivity against other receptors, unfavorable physico-chemical and PK properties, or intellectual property issues. These changes can be handled by de novo design tools. There are many examples in which computational de novo design has proved useful in generating novel ideas[1–3] but often lack of synthetic feasibility prevented adequate evaluation.[4]

Current de novo design methods are primarily applied when the receptor structure is known and indeed there are many proprietary and commercial applications for this purpose (e.g., Refs. 5–10). However, there have been relatively few attempts to apply de novo design in drug discovery in the absence of the receptor 3D structure. The major challenges in this latter process are (1) capturing relevant information from the ligands alone for the design of novel structures, (2) ensuring that the generated structures are diverse, given the often limited diversity of the input (training set) structures, (3) ensuring that the computer-generated structures are useful in drug discovery, that is, that they have the required drug-like properties, are synthetically feasible and amenable to optimization. To address the extraction of ligand information, simulated receptors[11–13] and similarity-based methods[14–16] have been applied. The applied de novo design algorithms usually operate either using atom-by-atom or fragment-based construction. The principal objective of the former approach is to generate maximum diversity, whereas the latter aims to reduce the combinatorial explosion and generate synthetically more accessible structures. Some newer algorithms use a mixture of the two,[17] potentially incorporating the benefits of both atomistic and fragment-based methods.

In many earlier cases, de novo design was applied to generate a series of molecules that were subsequently ranked or filtered using in silico models. In contrast, the philosophy of the de novo design algorithm applied in this work is such that molecules are evolved in a

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process optimizing their fitness with regards to all available models and requirements. This provides a tool to perform inverse QSAR (i.e. only generate molecules that satisfy the QSAR model), as well as an efficient tool to sample relevant chemical space. Additionally, with the applied chemical transformations and build-up process, almost any feasible molecule could theoretically be produced, although due to the size of available chemical space, the molecules generated in a practical process will represent only a tiny fraction of what is possible. Our objective in this work was to devise ways of applying this new paradigm in our drug discovery process.

2. Results and discussion

Typically, de novo design is applied either to generate entirely novel structures ('from scratch'), or to modify existing molecules. In this work, both options were explored. The strength of our approach was that molecules were directly optimized in order to fit necessary models and property criteria rather than simply generating a large number of structures followed by virtual screening. The application of this approach is demonstrated using two case studies.

2.1. Case study 1. Scaffold hopping to generate novel selective norephinephrine re-uptake inhibitor (SNRI) ligands

In this case study five literature compounds, shown in Figure 1, were used as the training set. In the de novo design process 1000 molecules were generated using structural similarity (MACCS) and 1200 with the simultaneous use of 2D pharmacophore similarity (TGT) and properties (MP61) to drive the evolution of new structures. As shown in Table 1, the output structures showed great structural diversity (as shown by the MACCS fingerprints) among different runs but more similarity within each run. Also, as expected, generated molecules were less similar in comparison to the training set if pharmacophore/property similarity was used, hence this combination is preferable in lead-hopping exercises when significant structural changes are sought. Figure 1 also displays the 2D-pharmacophore (TGT), structural (MACCS), and property (MP61) similarities of these structures to the closest training set compounds. The MACCS similarity values indicate that some of the structures are novel and therefore potentially valuable from an IP perspective: for example, a Tanimoto value of 0.5 suggests that there is little in common structurally between the generated structure and the closest training set molecule. Interestingly, as shown in Figure 1, a number of known structures were reproduced in the output, the structures of which were patented by companies and not represented in the set of input compounds. The fact that structurally unrelated known actives were rediscovered in the de novo design process suggests that there is a good chance that some of the remaining structures might also be active hits; this is a commonly used 'proof of concept' in de novo design. More importantly, however, one of the top scoring compounds (structure not shown, TGT:1.00, MP61:1.00, as well as low structural similarity to the

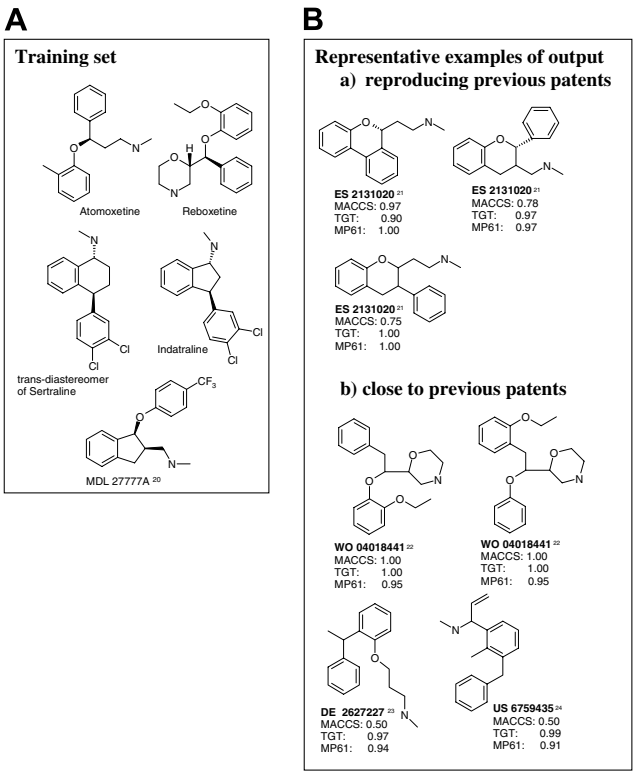

Figure 1. Case study 1: de novo design applied for scaffold hopping to generate novel selective norephinephrine re-uptake inhibitor (SNRI) ligands (A) the training set (B) representative examples for generated structures that closely resemble previously patented molecules (note that the molecule in DE 2627227 had been patented as an antidepressant without any specified molecular target). The highest Tanimoto structural (MACCS), 2D pharmacophore (TGT), and property (MP61) similarities to the five training set compounds are indicated below the structures. One of the best virtual hits (structure not shown, TGT: 1.00, MP61: 1.00, MACCS 0.66) was selected as the lead compound in the project. (See above-mentioned references for further information.)

training set compounds: MACCS:0.66) was found to be highly active and was selected as lead compound in the project at Neurocrine. Although the structure of this molecule cannot be presented here, this result is the ultimate success and validation of the design method.

2.2. Case study 2. Exploring optimal sidechain selection for the gonadotropin releasing hormone (GnRH) receptor

This was a practical case study, applied in our GnRH discovery program. Before this work was initiated, a completely novel and promising core had been selected from virtual screening a combinatorial library using our newly developed consensus scoring method.[18] The objective in this current study was to explore substituent space on this core at two positions to identify substitutions consistent with both the pharmacophore patterns present in the training set and the desired properties but without any prior knowledge of SAR on the scaffold. (SAR on this core was subsequently developed and almost 600 molecules were later synthesized and tested, among them those described in this study, as part of the medicinal chemistry program.)[19] Thus the core of the molecule was kept un-

changed and de novo design was applied to vary the substitutions to optimize the similarities of 2D pharmacophore (TGT) and property (MP61) fingerprints and simultaneously satisfy some other property constraints. MACCS fingerprinting was avoided in this example because it preferentially leads to already known substitution patterns. For consistency, the training set applied for de novo design was identical to that used in the virtual screening[19]: a diverse set of 100 GnRH actives (K_i < 100 nM) from the literature, patents, and Neurocrine's proprietary compounds (see Fig. 2). Structures were evolved from the molecular core with only hydrogens at the two substitution points of interest. In this process, ~10,000 molecules were generated. One major issue in this process was that a large number of structures were generated with substitutions at only one of the two possible positions. This arose due to probabilistic reasons, as the core itself was not allowed to be modified and more mutations are possible on a previously substituted sidechain than on one consisting solely of hydrogen. This effect was not compensated for in the scoring function; instead the set was filtered using a 3D pharmacophore, originally developed for virtual screening purposes.[19] This pharmacophore contained five feature points and an exclusion sphere, generated in MOE, with only those molecules containing at least four of these features retained (see Fig. 1 in Ref. 25). In pharmacophore screening, a maximum of 300 conformations for each molecule, as generated by the conformer import module in MOE, were passed through this 3D pharmacophore. (Although it would have been possible to use the 3D pharmacophore directly to drive ligand generation, this was not done for reasons of efficiency, as it would have necessitated conformer generation for over 100,000 ligands in every run.) Altogether 655 satisfactory molecules were generated and presented to the chemists. The majority of these molecules appeared to be chemically reasonable. Nonetheless, the chemists recommended changes to remove features that would have caused significant synthetic complications but were not believed to be necessary in light of the applied models. These changes were minor (the resulting structures had over 99% MACCS similarity with the original ones and satisfied all models) and included the removal of certain double bonds,

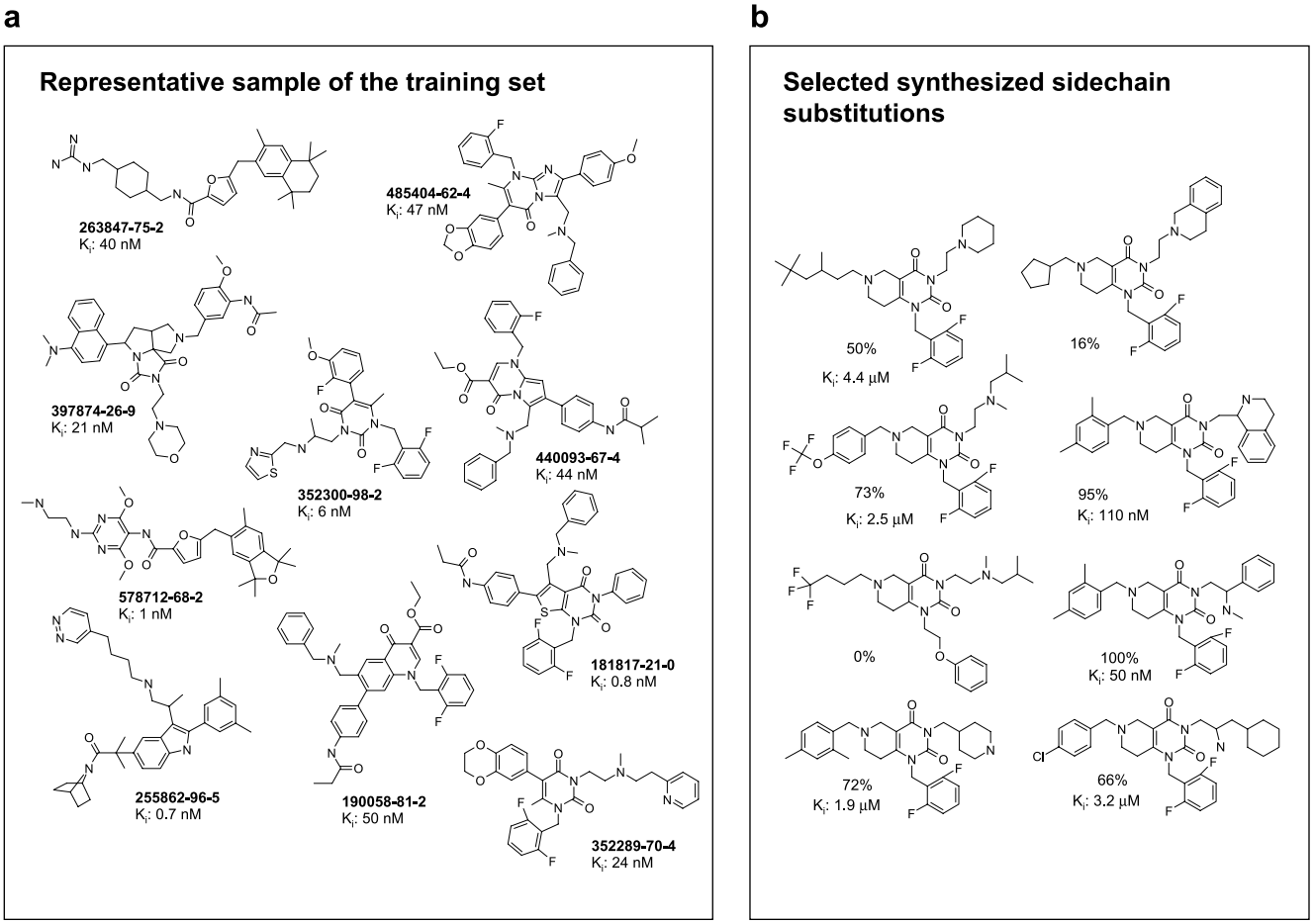

Figure 2. Case study 2: de novo design applied for exploring substitutions at two points that satisfy the 2D pharmacophore/property model for the gonadotropin releasing hormone (GnRH) receptor (a) representative examples of the training set, identified using their CAS numbers, with activities (K_i) of the displayed compounds ranging from 0.5 nM to 50 nM, (b) examples of synthesized sidechains generated for a novel core after minor modifications (such as removal of unnecessary double bonds, aromatizing or fully saturating rings, removal of certain heteroatoms) by medicinal chemists. Molecules with these changes still fit the in silico models but have improved synthetic accessibility. The percentages under the structures indicate the level of inhibition of the GnRH receptor at 10 μM concentration. The most active compound among these had a K_i of 50 nM. Further details on the synthesis and biological assay of these molecules are given in a separate publication.[19]

aromatizing or fully saturating rings and removing of selected heteroatoms from the molecule. This was necessary as the chemical space containing all molecules that satisfy the models is vast and therefore the particular molecules generated were considered only as representations of certain structural classes. The alternative solution would have been to run the process through many more iterations and filter the generated molecules to obtain ones with the predefined qualities but instead, the de novo design process in this study was employed as an idea generator and an improved set of substitutions was generated manually relying on the expertise of experienced medicinal chemists. Thirteen molecules were synthesized based on these ideas[19]: eight with the original substitution patterns from de novo design (shown in Fig. 2) and another five with different combinations of these substitutions. None of the recommended or actually synthesized substitutions were present in the training set (and the core was also different from those in the training set). Figure 2 also displays the percent inhibition of the synthesized molecules, measured at 10 μM concentration, the majority of which appears to have some binding affinity to the GnRH receptor (with the most active compound among these having a K_i of 50 nM).[19]

3. Conclusions

A novel approach for ligand-based de novo design is described in this article. The commercially available de novo design engine used in this work combines atomistic and fragment-based build-up with a wide range of structural mutations, hence it can produce highly diverse yet synthetically feasible solutions. It was shown that the evolutionary algorithm could be driven efficiently using a fitness function that combines aspects of both a computational model (such as pharmacophores and/or similarity) and medicinal chemistry (such as avoiding undesirable substructures, having the right molecular weight, number of stereocenters, log *P*, and other properties). As a result of this architecture, the majority of structures generated by the process after convergence satisfy all of the requirements present in the fitness function.

The application of this process was demonstrated on two examples: one showing the ability to do scaffold hopping, the other to find optimal substituents for a novel scaffold with no previous structure–activity data. It is important to recognize that automated algorithms for scaffold hopping and especially virtual testing of different sidechains are in widespread use in the industry, operating mainly through combinatorial library enumeration. However, such algorithms fundamentally differ from the ones described here, whereas combinatorial library-based algorithms blindly generate solutions using 'preconceived' substitution patterns, de novo design as described here drives the process through a highly diverse set of substitutions toward the optimal ones. In combinatorial libraries the number of considered sidechains must be limited to avoid combinatorial explosion, whereas de novo design could, in principle, generate any substitution pattern, many of which might not be easily anticipated based on the existing active compounds.

Due to the stochastic nature of the algorithm, every run appeared to generate different solutions (the duplicate rate was very low), with the population of structures within a run representing some structural diversity for the given solution class. It is important to recognize that even though molecules need to satisfy a number of requirements, the available chemical space is vast and thus the particular exemplars presented by the program after a few runs may not be the synthetically most attractive solutions, even though they are synthetically feasible. In this regard, de novo design can be considered as an idea generator: the resulting molecules are known to satisfy the computational models and can be used to propose highly similar but perhaps more accessible molecules (easier synthesis, availability of starting materials or intermediates). It is likely that once more experience is gathered about this thought process, some of the reasoning can be added to the scoring function to further improve the results.

4. Methods

A schematic diagram of the de novo building process is displayed in Figure 3. The Evolutionary Algorithm Inventor (EAI),[17] developed by the Pearlman group and currently distributed by Tripos, was applied as the de novo design engine. The program uses an evolutionary algorithm to generate new chemical structures from a seed set or previous generation and can be used in conjunction with any single or composite external scoring function (the scoring functions applied in this work are described below). Evolutionary algorithms evolve improved populations of structures by performing modifications on the members of previous generations ('mutations') and swapping successful substructures among different molecules ('crossovers'). In this process, molecules evolve via 'survival of the fittest' so as to optimize the applied scoring function. The mutation/crossover operators all have adjustable probabilities that describe the frequency of different changes on the molecules, such as adding or deleting atoms, changing their type, hybridization state, or charge, adding certain groups or changing ring sizes. In this work, the probabilities of these mutations were changed substantially

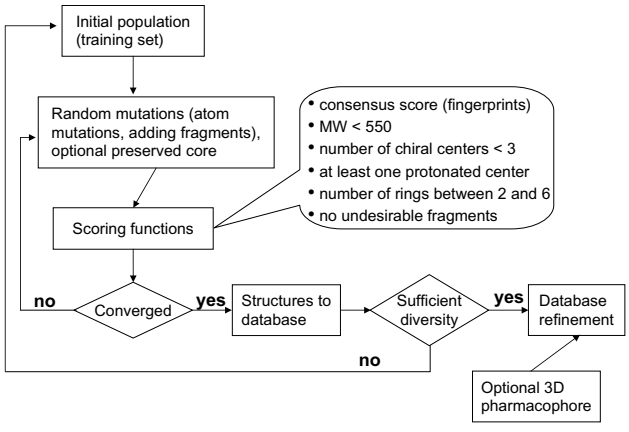

Figure 3. Schematic diagram of the de novo design process.

from their default values to reflect the intuition of the chemists in terms of synthetic accessibility and to remove certain undesired substitutions. The populations of structures generated in the process are chemically plausible. The charge operator was entirely turned off, as molecules were protonated/deprotonated appropriately in the scoring step, as described below. Due to the stochastic nature of the applied algorithms, molecules generated in different runs have significant differences in their structures, whereas members of the final population in each run usually provide multiple examples for a given structural class (see Table 1 for practical examples). Thus, in order to gain high structural diversity in the solutions, the population size was kept low (50–100) and a large number of separate runs were carried out. Although using smaller populations may result in a larger number of generations being required before the termination criteria (e.g., that at least 60% of the population has a total score within 2% of the theoretical maximum) are met, the length of time taken to process each generation is directly proportional to the population size. Thus multiple runs with low population sizes generally take only slightly longer than fewer runs with larger populations but have the advantage of generating a more diverse set of solutions. It must be noted that these termination criteria are unusually stringent and were created with the hope of identifying really good molecules. The extent of coverage of the search process along with number of iterations required to reach a suitable termination criteria had previously been checked varying both the initial population and mutation/crossover operation probabilities and from this, suitable parameters for practical de novo design were selected (data not presented). The goal of this current work was to obtain further validation on examples directly relevant in drug discovery.

Table 1. Structural diversity (MACCS) of the generated structures [a]

System[b]	Internal (single run)	Internal (all runs)	External (all runs)
SNRI -MACCS	0.97/0.98	0.75/0.80	0.92/0.96
SNRI-TGT/MP61	0.75/0.72	0.55/0.53	0.54/0.53
GnRH-TGT/MP61	0.94/0.94	0.81/0.81	0.83/0.83
GnRH-TGT/MP61/3DP	n.a.	n.a.	0.86/0.86

[a] Internal diversities were calculated as the mean/median of the maximum pairwise Tanimoto similarities of the MACCS fingerprints of the molecules generated in the de novo design process. External diversities were calculated in comparison to the training set and were the mean/median of the maximum pairwise Tanimoto similarity values of the MACCS fingerprints between the training set and the structures from de novo design. The acronyms SNRI and GnRH refer to the targets selective norephinephrine re-uptake inhibitor and gonadotropin releasing hormone receptor, respectively see text for further details.

[b] The calculated diversities pertain to the following receptors and fingerprint scoring function element: selective norephinephrine re-uptake inhibitor ligands generated using MACCS fingerprints (SNRI-MACCS) and combination of TGT/MP61 fingerprints (SNRI-TGT/MP61), the gonadotropin releasing hormone receptor generated using TGT/MP61 fingerprints (GnRH-TGT/MP61) and the same output filtered using a 3D pharmacophore (GnRH-TGT/MP61/3DP). Apart from the above fingerprints, the scoring function also contained a number of properties. See text for further details.

Molecular structures were manipulated and scored using the MOE software suite and its batch version.[25] Each population of structures was transferred from EAI to MOE as a set of SMILES strings via a Python wrapper. After hydrogen addition, molecules were protonated according to the rule-based system implemented in MOE,[25] reflecting the protonation state in an aqueous environment. They were then scored using a scoring function that included Tanimoto fingerprint similarity to a set of active reference structures using either MACCS[26] or an unweighted combination of TGT[25] and MP61[27] fingerprints, all as implemented in MOE. These fingerprints provide reasonable representations of structural, 2D pharmacophore and property similarity, respectively. The scoring function was developed over time together with a group of experienced medicinal chemists and incorporates elements specifically for this work as well as more general factors included in other discovery projects such as a long list of undesirable chemical substructures. These functions comprised of the product of individual scores derived from the following components: fingerprints, molecular weight, stereochemistry, undesirable fragments, cationic presence, and ring count. The fingerprint score itself included the sum of the maximum similarity values of each of the fingerprints applied. The molecular weight and stereochemistry scores were functions with continuously decreasing values above the target value (MW above 400, number of stereocenters above 1). The cationic ring and unwanted center scores each were 1 if the condition was satisfied (i.e. the molecule included at least one atom with a formal positive charge, the number of rings was between 2 and 6 and contained no undesirable substructures) and 0.9 if it was not. In order to remove compounds that would be difficult to synthesize, different methods for assessing synthetic accessibility were considered.[28] For the sake of speed, the final method selected was a simple list of undesirable substructures combined with counts of the number of stereocenters and rings. In addition, the undesirable substructures list was used to penalize compounds containing any of a list of reactive and non-drug-like fragments from the literature[29] and almost as many new entries added after consultation with medicinal chemists (defined by a total of ~60 SMARTS strings). Due to the 2D nature of the applied scoring functions, the absolute stereochemistry of the compounds was ignored. If necessary, this can be taken into account using 3D methods, such as flexible alignments and pharmacophores either as part of the scoring functions—which would drastically slow the process—or as a post-processing step. However, since most compounds were usually synthesized as racemic mixtures the first time, this was not deemed necessary. The entire scoring function was empirically optimized for the speed of generating structures, as well as for the diversity and quality of output acceptable to a panel of medicinal chemists. Initially, the starting molecules set was varied to establish whether diversity and the speed of generating 'acceptable' structures are dependent on it. It was found that the diversity of resulting structures was not dependent on the seed set, even when one started from, for example, ethane, as opposed to a varied set of drug-like cores, as long as

the starting structures were sufficiently far from being acceptable solutions themselves. However, in terms of efficiency (number of steps required to reach convergence), from all the starting points studied the best was a set of 100 most frequently occurring cores extracted from the CMC[30] database in this work. In the second case study, the task was to vary the substitution on a scaffold, the structure of which was kept unchanged during the de novo design process and thus this core was used as a starting point in the build-up process.

The generated molecules were collected in a MOE database and duplicates were removed. Despite the stochastic nature of the molecule generation towards a common goal, there were fewer than 1% duplicates which shows the vastness of the search space satisfying the similarity/pharmacophore/property criteria accessible by the de novo design process.

The entire process shown in Figure 3 was driven using a shell script that iteratively called the de novo design engine for structure generation, which subsequently called MOE/Batch for scoring. In runs using structural (MACCS) similarity, convergence was typically achieved within 300–1000 generations, whereas up to 1500–2000 generations were typically required to get good convergence using pharmacophore/property (TGT/MP61) similarity. (The above difference in the number of steps required to achieve convergence is likely to be due to the fact that in drug-size molecules the TGT fingerprint contains a significantly greater number of bits than the MACCS fingerprint.) The time required scaled almost linearly with the number of generations and for molecules in the SNRI case study took about 2 h to run through, 1000 generations on a 2.8 GHz Xenon PC with 2 GB of memory, running under the RedHat Enterprise 3.0 operating system.

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