NovoFLAP: A Ligand-Based De Novo Design Approach for the Generation of Medicinally Relevant Ideas

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NovoFLAP is a computer-aided de novo design tool that generates medicinally relevant ideas for ligand-based projects. The approach combines an evolutionary algorithm (EA-Inventor) with a powerful ligand-based scoring function that uses both molecular shape and pharmacophore features in a multiconformational context (FLAP). We demonstrate that NovoFLAP can generate novel ideas that are not only appealing to design scientists but are also validated by comparison to compounds known to demonstrate activity at the desired biological target. NovoFLAP provides a novel computer-aided design technique that can be used to generate ideas that maintain desirable molecular attributes, such as activity at the primary biological target, while offering opportunities to surmount additional design challenges. Application to the design of the first nonbasic $5HT_{1B}$ antagonist is presented.

INTRODUCTION

Drug discovery is a challenging, multidimensional problem in which many molecular characteristics are simultaneously optimized. These could include factors as complex and diverse as in vitro and in vivo activity at the desired target, desirable absorption properties, suitable distribution, metabolism, and elimination (ADME) profiles, avoiding molecular toxicities and ensuring opportunities for obtaining intellectual property. During the course of a discovery program, it is not unusual to attain some of these desired characteristics in a chemical series and to have others that still require improvement. Project scientists apply a wide variety of design approaches in an attempt to maintain the desirable characteristics while improving upon those properties that do not yet meet expectations.

Virtual screening has become an increasingly popular way to computationally approach the problem of finding new chemical structures for a given biological target. When the three-dimensional structure of the biological target is known, virtual screening most often involves computationally docking a selected set of test compounds to the active site of the biological target and scoring the docked structures in order to identify those compounds with greatest potential.[1] Such detailed structural information is, unfortunately, not currently available for many, if not most of the important targets in drug discovery. For these more typical projects, virtual screening relies on reference compounds and uses a variety of searching algorithms that attempt to locate similar compounds in a database of chemical structures. The hope is that similar compounds identified in this manner will exhibit desirable biological characteristics while also offering opportunities to address those difficulties facing the current design effort.

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A virtual screening campaign may be limited in scope to considering available compound collections, or it may involve assessing a virtual library of compounds that could potentially be synthesized. For virtual libraries, both the creativity applied to the design and the practical limitations that prevent considering more than a subset of medicinally reasonable chemical possibilities[2] are both limiting factors.

De novo design approaches, where novel chemical entities are designed computationally, offer an additional approach to the generation of new molecular candidates with potential to overcome design challenges. Since structures are computationally generated, one is not limited to previously known or enumerated chemical entities. In addition, one is substantially less dependent upon the experience base and creativity of the individuals involved in compound design. De novo design comes with its own set of challenges, however, including the requirement to design chemically reasonable, medicinally meaningful ideas. Considerable attention has been paid to the development and application of de novo design approaches in structure-based design,[3] where the three-dimensional structure of the biological target is known. Applications of de novo design to ligand-based drug discovery problems have been less extensive.[4]

Because of the potential utility de novo design approaches in the ligand-based area would offer and the recent emergence of a number of scientific and technological capabilities, we felt that a unique opportunity existed for an advance in this important scientific area. This paper details our recent efforts in ligand-based de novo design and the validation of our approach in projects of topical interest in Medicinal Chemistry.

Requirements for an Effective Ligand-Based De Novo Design Approach. Desirable elements of a computer-aided de novo design approach include: (1) The ability to generate medicinally meaningful new structures for consideration; (2) The ability to computationally evaluate or score the designed structures to identify the best possibilities; and (3) The

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continual improvement in de novo designed structures based on feedback from this computational scoring.

The first of these, meaningful structure generation, has been a significant challenge for de novo design approaches. To contribute effectively to drug discovery, the generated structures must meet much higher standards than merely obeying simple rules of chemical valency and rudimentary stability. To be considered viable, design ideas need to meet the more stringent criteria of medicinal chemistry relevance, which includes factors, such as functional group content, molecular size, complexity, and synthesizability, etc. Further complicating this issue, while some medicinally relevant characteristics are universally desirable (e.g., no highly reactive functionality), there are others for which what is acceptable or even desirable can vary depending on the goals and objectives of the discovery project as well as the individual scientists performing the evaluation.[5] To be applicable to pharmaceutical design, it is important for a de novo design algorithm to comply with these medicinal chemistry expectations and to be tunable to variations on these expectations as relevant to the project at hand.

Computationally scoring the de novo designed structures can also be a considerable challenge. When faced with the problem of developing new idea structures, a project team may have extensive information on numerous compounds from a series or information could be as limited as a single compound disclosed by a competitor or a singleton hit obtained in a high-throughput screen campaign. Compounds may all act at the same target site, exploiting the same recognition features, or, as is known for several targets of medicinal interest,[6] there could be multiple binding modes exploiting different sets of recognition features. An effective scoring algorithm for de novo design needs to be able to cope with each of these challenges in order to ensure wide applicability of the approach.

Based upon scoring feedback on de novo designed structures, a structure generation algorithm should ideally have the capabilities of suggesting even better (higher scoring) ideas in future iterations. Such an evolutionary approach to de novo design would be expected to optimize along a path of continually improving design options for consideration by the project team.

Ligand-Based De Novo Design: Computational Methods. The ligand-based de novo design developed and described herein involves a combination of an evolutionary structure generation engine, EA-Inventor,[7] and a proprietary, validated, ligand scoring approach developed at AstraZeneca, Flexible Ligand Alignment Protocol[9] (FLAP).[8] As will be demonstrated below, this powerful combination meets the requirements outlined above for a viable ligand-based de novo design approach.

De Novo Structure Generation: EA-Inventor. Evolutionary Algorithm Inventor (EA-Inventor) is a structure generation program that offers a unique capability, meeting the de novo design requirements outlined above. EA-Inventor is based on an evolutionary algorithm that operates on an initial population of structures to invent new structures with improved scores.[10] EA-Inventor is unique in that it has the ability to work with any user-defined scoring function that provides results in the form of a numerical evaluation of designed structures. The design process most typically begins with a set of user provided structures, which are considered the initial population. This initial population is structurally modified by EA-Inventor, and the new set of structures, termed the first generation, is submitted to the scoring function for evaluation. Only the structures with good scores are retained and modified by EA-Inventor to form the second generation. This process is repeated until the last generation is reached. In order to generate new structures, EA-Inventor contains a fragment library with over 1300 fragments extracted from the Molecular Design Limited Drug Data Report (MDDR).[11] These fragment structures[12] are the structural sources of EA-Inventor's de novo design. Thirty-two chemical transformation operators also enable the generation of valid chemical structures obeying valence rules. EA-Inventor has the capability of generating large numbers of structures and can, therefore, work with large populations as part of the design process. In addition, substructural components, such as molecular cores, can be preserved during structure modification. This is particularly useful, for example, for designing new R-groups or scaffolds, while other molecular components are preserved.

Scoring of De Novo Designed Structures: FLAP. The scoring algorithm used for structure evaluation in this work grew out of efforts at AstraZeneca in ligand-based virtual screening. Compounds are scored against either a reference compound or a set of reference compounds, and scores are determined based on the fit of overall molecular shape and pharmacophoric features to these reference compounds. Each molecule in the reference set is conformationally expanded using either Omega[13] or LigPrep,[14] within generous but reasonable energy distributions, and each conformation of each structure is compared individually to conformations of the compounds to be scored. Each molecule receives a shape match score using ROCS[15] and a pharmacophore alignment score using the proprietary AstraZeneca program Triphic.[16] The shape and pharmacophore scores are then used to form an aggregate composite score for each conformation of the designed molecule. Shape and pharmacophore scores are normalized and summed with equal weightings for a composite maximum score of 2.0. The best scoring conformational match with the reference structure(s) is retained as the score for that compound. When the score for a compound exceeds a predefined cut off (typically ≥ 1.4), it is considered a hit and are retained for further consideration. All options are under user control in FLAP, including the ability to use even a single conformation of a single compound as the reference should sufficient information be available to scientifically justify such an approach.

It should be noted that the FLAP approach avoids the need to determine whether a set of reference compounds bind to the target receptor by exploiting the same recognition features. Since each conformation of each reference structure is evaluated in scoring, multiple pharmacophore patterns can, in principle, be exploited in a single FLAP evaluation. Thus, there is no need with FLAP to pursue the tedious and potentially unproductive or even misleading activity of developing a unified overlay pharmacophore model for a series of active compounds. A hit on any of the conformations of individual compounds used in the reference set is sufficient, irrespective of whether the reference compounds share a similar pharmacophoric pattern.

We have used FLAP in ligand-based virtual screening campaigns in AstraZeneca and have identified compounds

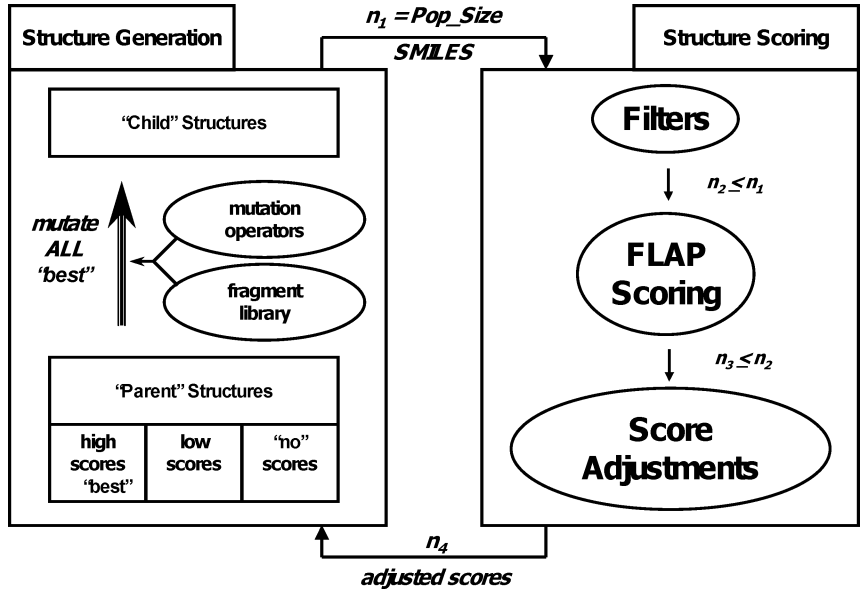


Figure 1. Schematic representation of the NovoFLAP design cycle. The design cycle begins with a defined initial population that is first submitted to a set of filters (top right), removing compounds based on their chemical features. These compounds are then submitted to scoring by FLAP. FLAP scores are then adjusted based on the two-dimensional similarity to the compound(s) that are being scored against with a penalty imposed for structures that are too similar. Surviving compounds with high scores are "hits" for the first generation. These compounds are mutated to form child structures that constitute the initial population of generation two, and the process continues through the desired number of generations.

that have confirmed in project assays with a success rate of between ca. 20 and 80% (averaging ca. 25%).[17] Given this performance level in virtual screening, we felt that this approach offered a good possibility as a scoring approach for exploitation in de novo design.

NovoFLAP: An Integrated Ligand-Based De Novo Design Approach. NovoFLAP is the integration of the structure generation and evolutionary design tool EA-Inventor, with the validated scoring capability of FLAP. A flowchart of the design cycle for NovoFLAP is shown in Figure 1.

A design cycle begins with an initial population of chemical structures. At the beginning of a design run, initial populations can be user defined and can be either as targeted as the reference compounds themselves or as liberal as a random population of compounds selected by EA-Inventor. A set of chemical filters is then applied to remove those compounds that are considered undesirable from a medicinal chemistry perspective. Our chemical filter is based upon a set of undesirable chemical features as defined by Astra-Zeneca Medicinal Chemists. This is supplemented with a list of structural features encountered in early designs with NovoFLAP that, while chemically reasonable, were deemed to be medicinally undesirable. Compounds surviving the filter are then submitted to FLAP where they are conformationally expanded with each conformation submitted to scoring. We have typically had good success scoring against one compound with known desirable pharmacological properties. To prevent the evolutionary process from simply designing the compound being used for scoring, a score adjustment is added that imposes a penalty based on the two-dimensional fingerprint[18] similarity of the designed compound to the compounds being used for scoring. The penalty function is a linearly scaled multiplicative factor applied between similarities of 1.0, when the scaling factor is 0.0, causing an exact fingerprint match to receive a score of 0.0, to a similarity of 0.6, at which point the scaling factor reaches 1.0. This penalty has the effect of pushing designs away from the compound being scored against based on two-dimensional fingerprint similarity, while the FLAP scoring rewards similarity to the scoring compound based on three-dimensional shape and pharmacophore alignment. For the following generation, high-scoring compounds are mutated using EA-Inventor's set of chemically meaningful operators to generate a new population of structures that are similarly filtered and evaluated. EA-Inventor continually identifies desirable structural modifications and exploits these as future generations are constructed. With our scoring function, a typical design involves approximately 25 to 50 design generations with an initial population of 25 to 50 molecular structures. Because of the random component of the evolutionary design algorithm, rerunning a design may give very different design results. Here, it is not unusual to find that certain designs yield results that are preferred.[19] For this reason we often will perform design runs in triplicate. To facilitate evaluation of the output, designed compounds meeting the scoring cut off are displayed in pdf format along with mol2 files containing the three-dimensional overlays between the designed and scoring compounds. We have found that it is useful to inspect all ideas from all generations in the design process in addition to those compounds obtaining the highest overall scores.

Success Criteria and Validation of the NovoFLAP Approach. Evaluation of ligand-based de novo design ideas can be more challenging than evaluating hits from collections of available compounds. With a virtual screen hit from a collection of available compounds, one can obtain a sample that can be submitted to an appropriate screen and immediately determine the accuracy of the prediction. For de novo design, however, there is no reason to expect that the compounds will be previously known, more-or-less readily available for testing. We, therefore, spent considerable effort

developing a mechanism for scientifically validating the NovoFLAP approach and demonstrating that medicinally meaningful results could be obtained.

The success criteria we applied for assessing NovoFLAP were three-fold: (1) New, chemically meaningful ideas should be generated that are of interest to design scientists; (2) Starting with at least one example from a reference series, we should be able to generate new ideas within that series; and (3) Starting with at least one example from a reference series, we should be able generate new chemical series that also demonstrate activity at the biological target. We are pleased to report that NovoFLAP has been successfully validated by all three of these criteria.

The last two success criteria depend on being able to confirm that the NovoFLAP designed compounds demonstrate an appropriate level of biological activity at the target of interest. This is a challenge, since biological information will not currently exist for most de novo designed compounds. Unlike in virtual screening, it is, therefore, not possible to determine a direct success rate for de novo design. To address this issue, we took the set of compounds designed by NovoFLAP in each of the studies reported below and compared these structures to publicly disclosed compounds that are known to be active at the desired target. Sources of information included the primary literature, patents, and the MDDR. Because of the random, evolutionary nature of the NovoFLAP process, one cannot be assured that identical or even similar structures to publicly disclosed compounds will result in a design. The observation of designed molecules with very similar structures, however, supports the validity of the overall design approach. While we provide examples of these types of comparisons in the following sections, it is important to remember that NovoFLAP provides many ideas and that for validation purposes we have only presented select examples that can be related to compounds that are known to be successful at the biological target of interest.

For each of the studies reported below, between three and five designs using 25 generations of 25 structures were performed. Each design run generated approximately 100 ideas meeting the cut-off criteria for scoring (see above). As is the nature of a random evolutionary process, when, i.e., which generation, in the design process these structures were obtained varied. Empirically, we have found that it is useful to perform the same design experiment repeatedly (e.g., three times) since this random aspect can often result in different outcomes from the same starting conditions.

NovoFLAP De Novo Design Results. *Neurokinin 1 (NK1).* The search for NK1 antagonists has been active in the pharmaceutical industry for some time with therapeutic targets that have included depression, asthma, nausea, and analgesia.[20] A variety of chemical series have demonstrated significant potency at this targeted receptor. As a first test of the NovoFLAP approach, we selected a well know NK1 antagonist, CP99994 (Figure 2, $IC_{50} \sim 0.5$ nM),[21] as a starting point for de novo design efforts. Using NovoFLAP to design novel chemical entities, a variety of medicinally interesting compounds were generated. These designed compounds were then inspected to see how they compare with public domain compounds that are known to have NK1 activity.

In addition to a variety of interesting, medicinally relevant designs, there are two notable compounds that emerged from this study that are shown in Figure 2 (**1a**, **2a**). The first structure (**1a**) replaces the secondary amine in the core of CP99994 with an ether functionality. This is a small structural change that might be expected to alter physiochemical properties within a CP99994-like series. An ether linkage at this site is known in many NK1 actives, and 32 examples of NK1 antagonists with ether linkages at this point can be found in the MDDR. The Merck compound L-733060 (**1b**, K_i[22] $\sim$ 200 pM) is a specific example that contains this structural motif.

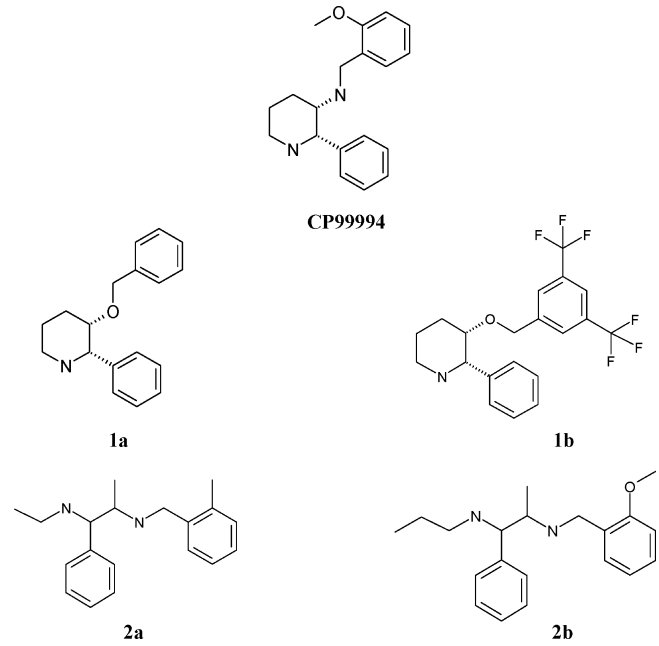
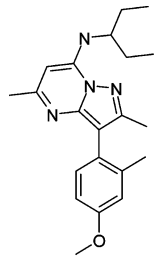


Figure 2. Starting compound CP99994, compounds designed by NovoFLAP (**1a**, **2a**) and known compounds with similar structures (**1b**, **2b**).

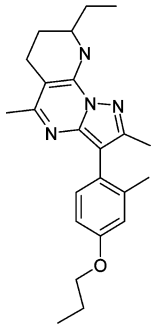
NovoFLAP also designed the ethylene diamine compound **2a** (Figure 2). This ring-opened derivative represents a significant change from CP99994 that would be considered by most to be a change in chemical series. Members of the ethylene diamine series are known to exhibit NK1 activity, and this structural class has been the object of Pfizer patents. One specific example from this series is shown in Figure 2 (**2b**).[23] While structurally more complex than example **2b**, AstraZeneca has also made numerous compounds belonging to this class that are potent (single digit nM) NK1 antagonists.[24]

Corticotrophin Releasing Factor (CRF). Corticotrophin releasing factor (CRF) is a major mediator of the effect of stress, and CRF antagonists are targets for treatment of anxiety and depression.[25] One noteworthy J-domain compound is DMP904 (Figure 3, $IC_{50} \sim 1$ nM).[26] DMP904 was used as a starting point in de novo design by NovoFLAP, and a particularly interesting compound with an additional fused ring (**3a**, Figure 3) was suggested. A very similar compound with this fused ring template can be found in a patent from Eisai[27] (**3b**, $IC_{50} \sim 1.5$ μM).[27]

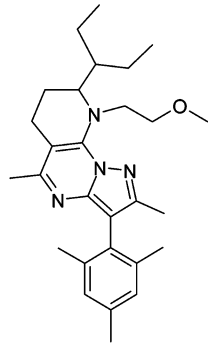
Angiotensin II (AII). The development of potent AII antagonists as antihypertensive agents has been the focus of considerable effort in the pharmaceutical industry, based, in part, on the hope that AII antagonists would not demonstrate some of the side affects associated with angiotensin converting enzyme inhibition.[28]



DMP904



3a



3b

Figure 3. Starting compound DMP904, compound designed by NovoFLAP (**3a**), and known compound with similar structure (**3b**).

Beginning with a compound reported by ICI[29] (Figure 4, $IC_{50} \sim 31$ nM), several de novo design experiments were conducted with NovoFLAP. Particularly noteworthy compounds from these designs can be found in compounds **4a**−**7a** (Figure 4). The first two examples, **4a** and **5a**, are designs that represent significantly different chemical series relative to the starting point. The pyrimidone **4a** is a structural type that is well-known in the literature as AII antagonists, with 84 examples in MDDR.[11] One specific example of this known structural class is the Merck compound **4b**[30] ($IC_{50} \sim$ 5 nM). Designed compound **5a** has one of the rings found in the quinoline of the ICI compound saturated and substituted with nitrogen, forming a tetrahydronaphtheridine. This chemical series can also be found in the patent literature[31] (**5b**).

Two additional designed compounds, **6a** and **7a**, involve changes in the terminal phenyl ring of the biphenyl tetrazole moiety. Designed compound **6a** replaces this phenyl ring with a pyridine, and **7a** replaces this ring with a thiophene. Exemplary structures with this type of modification have also been reported in the patent literature by Merck (**6b,**[32] $IC_{50} \sim 4$ nM, and **7b**).[33] Here it is interesting to note that the designed compounds, while meeting the criteria of the scoring function for this design, do not contain the tetrazole ring. Most likely, and as demonstrated by the literature compounds in many of these series, this tetrazole ring is required to maintain substantial potency at the AII receptor. This illustrates an important point about using de novo design methods. While it is possible that a compound coming from de novo design can be exploited directly, it is also reasonable to think of a quality de novo design tool as an idea generator. It is quite legitimate that the idea kernels generated be modified and improved, as is true for any developing idea progressed as part of the drug discovery process. Here, with the applied criteria, NovoFLAP suggested the pyridine and the thiophene as replacements. Further consideration of structure−activity relationship (SAR) requirements would modify these suggestions to include the tetrazole substitutent.

Antihistamines: H1 Antagonists. Antihistamines are widely used for the treatment of allergies, in particular, in response to the seasonal release of pollen, resulting in allergic rhinitis. Classical antihistamines are H1 antagonists.

Beginning with Benadryl ($K_i \sim 2.5$ nM),[34] a variety of compounds were designed by NovoFLAP that are very similar to compounds known to have activity at the H1 receptor (Figure 5). For example, cyclization to form a seven-membered ring is shown for **8a**, and this structural motif is found in Loderix[35] (**8b**), a potent H1 receptor antagonist. Six-member rings at this position are also suggested by NovoFLAP, and compounds like **9a** are also known to demonstrate H1 activity, with similar compounds found in the patent literature[36] (**9b**).

Application of NovoFLAP to Drug Discovery. *$5HT_{1B}$ Antagonists.* 5-Hydroxytryptamine 1B receptor ($5HT_{1B}$) antagonists have been the target of many drug discovery programs, as these compounds offer potential treatments for depression and anxiety.[37] The known $5HT_{1B}$ antagonists contain basic amines that are believed to interact with an aspartate on transmembrane helix 3 of the receptor.[38] In addition, $5HT_{1B}$ antagonists can be moderately lipophilic. This presents a design challenge for drug discovery since lipophilic bases have the potential to interact strongly with the hERG ion channel, which can result in undesirable cardiovascular effects.[39] In addition lipophilic bases may lead to phospholipidosis.[40]

In an effort to avoid these potential safety liabilities, we used NovoFLAP to find replacements for the basic piperazine moiety found in our $5HT_{1B}$ antagonist series.[41] Preserving the core structure of antagonist **10a** (Figure 6, pK_a 7.66 ± 0.05),[41] but allowing the piperazine region to change, NovoFLAP designed the pyrazole replacement, **10b**. For these designs the piperazine was modeled in the neutral form, since a neutral replacement was desired. While our typical approach of exploiting multiple design runs was employed, each generating ca. 100 ideas meeting cut-off criteria, the pyrazole replacement was identified in an early generation of one of the first designs. The pyrazole affords the opportunity to constructively interact with aspartic acid on helix 3, while being considerably less basic (expected pK_a approximately 3) than a piperazine, and was selected as the most interesting design concept out of the several identified in these studies. The pyrazole-based idea was exploited in several $5HT_{1B}$ antagonist series, with exemplary compound **11** (Figure 6, pK_a 3.28 ± 0.05),[41] demonstrating high affinity for the $5HT_{1B}$ receptor (0.43 nM) and desirable in vitro and in vivo activities. In addition, all tested pyrazoles had hERG IC_{50} values that were substantially improved over the range observed for the piperazine analogs, and no pyrazole had measurable EC_{50} values in our in vitro phospholipidosis assay.[42] Both of these observations are very desirable outcomes from a safety perspective.

This design effort illustrates a very useful application of the NovoFLAP paradigm as well as some of the practicalities of drug discovery efforts. Rather than explicitly synthesizing **10b,** the project team exploited the pyrazole replacement concept in what were considered to be more readily synthe-

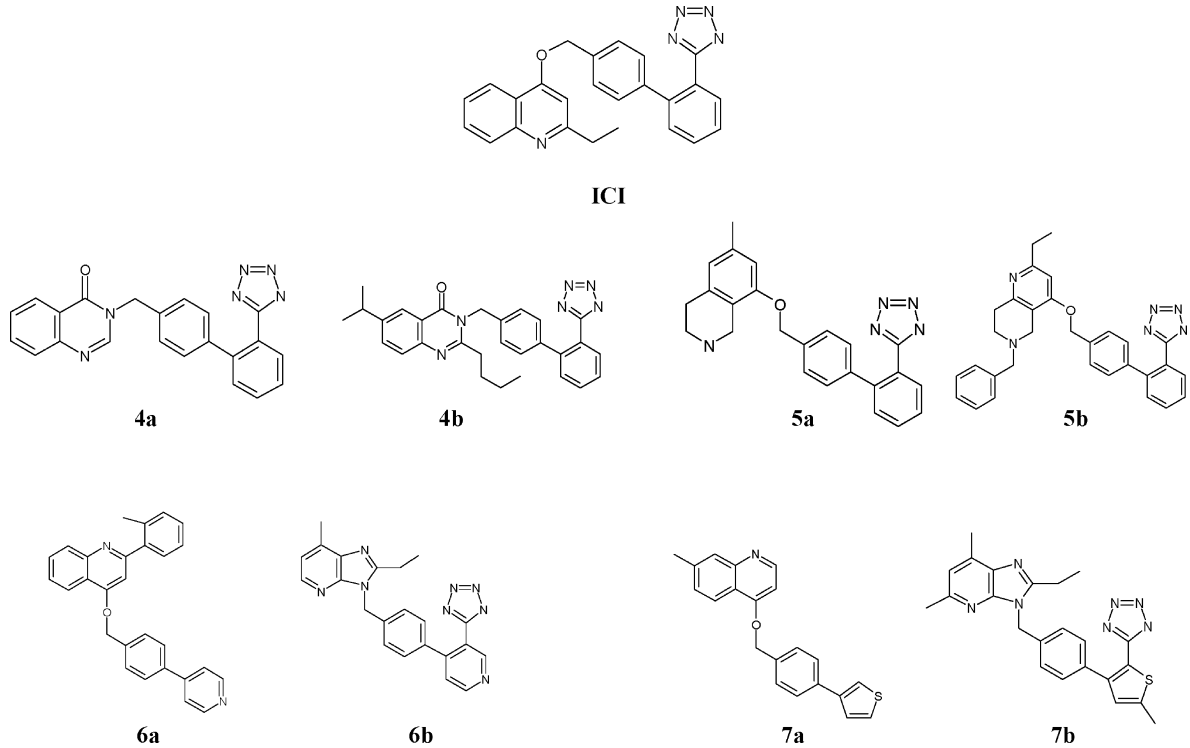


Figure 4. Starting compound ICI. NovoFLAP generated idea compounds (**4a**−**7a**) and known compounds with similar structures (**4b**−**7b**).

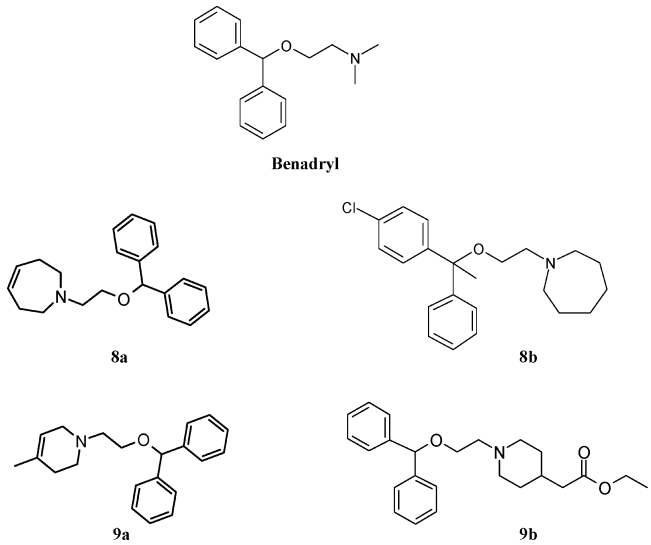


Figure 5. Starting compound Benadryl, compounds designed by NovoFLAP (**8a**, **9a**) and known compounds with similar structures (**8b**, **9b**).

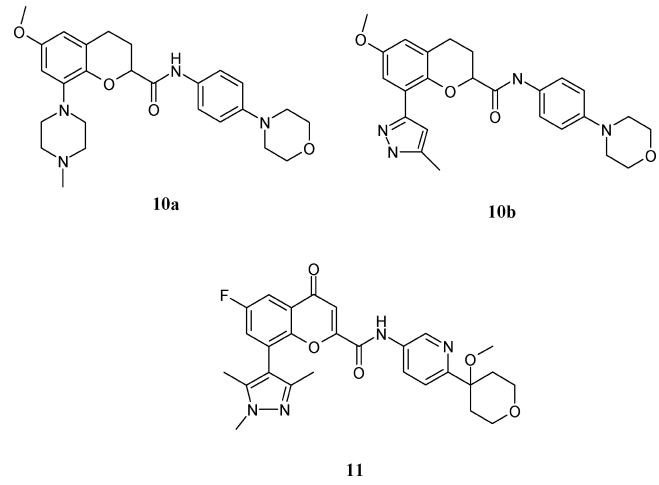


Figure 6. Piperazine $5HT_{1B}$ antagonist (**10a**), NovoFLAP designed pyrazole replacement (**10b**), potent, in vitro and in vivo active pyrazole-based $5HT_{1B}$ antagonist (**11**).

sizable derivatives, leading to many pyrazole derivatives demonstrating desired levels of activity and improved safety profiles.[41]

To our knowledge, this is the first demonstration that a basic amine, protonated at physiological pH, is not required for in vitro or in vivo activity at the $5HT_{1B}$ receptor.

SUMMARY

NovoFLAP combines the evolutionary design capabilities of Evolutionary Algorithm Inventor (EA-Inventor) with the scoring function Flexible Ligand Alignment Protocol (FLAP) to generate a novel de novo design capability for ligand-based discovery projects. We have demonstrated that NovoFLAP can generate ideas that are both appealing to design scientists and validated by comparison to compounds known to demonstrate activity at the desired biological target. Specific examples where NovoFLAP has generated either significant modifications of existing molecular frameworks or structurally new molecular templates relative to design starting points (i.e., lead hopping) have been provided, demonstrating the power of this new de novo design method.

NovoFLAP is currently being exploited in AstraZeneca as part of our overall computer-aided molecular design strategy.

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(18) Fingerprints are similar to those used by Daylight, Inc. and are generated using AZ proprietary software. Unpublished results Cosgrove, D.

(19) Historical evolutionary processes have similarly resulted in different but equally valid outcomes. For example, evolutionary processes beginning with primordial soup lead to dinosaurs. Following their extinction after millions of years of survival, evolutionary processes that depend upon the same building blocks lead to humans. While both outcomes are viable, we presumably feel that the latter was preferable.

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