ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Creation of Novel Cores for β-Secretase (BACE-1) Inhibitors: A Multiparameter Lead Generation Strategy

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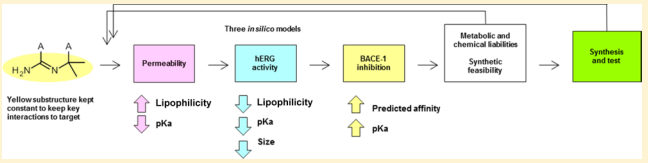
Supporting Information

ABSTRACT: In order to find optimal core structures as starting points for lead optimization, a multiparameter lead generation workflow was designed with the goal of finding BACE-1 inhibitors as a treatment for Alzheimer's disease. De novo design of core fragments was connected with three predictive in silico models addressing target affinity, permeability, and hERG activity, in order to guide synthesis. Taking advantage of an additive SAR, the prioritized cores were decorated with a few, well-characterized substituents from known BACE-1 inhibitors in order to allow for core-to-core comparisons. Prediction methods and analyses of how physicochemical properties of the core structures correlate to in vitro data are described. The syntheses and in vitro data of the test compounds are reported in a separate paper by Ginman et al. [*J. Med. Chem.* **2013**, *56*, 4181−4205]. The affinity predictions are described in detail by Roos et al. [*J. Chem. Inf.* **2014**, DOI: 10.1021/ci400374z].



KEYWORDS: *BACE-1 inhibitor, β-secretase inhibitor, fragment optimization, scaffold hopping, multiparameter optimization, core optimization*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a major unmet medical need.[3] One of the pathological hallmarks of AD is amyloid plaques in the diseased brain.[4] These plaques are largely consisting of amyloid-β peptides, which are produced by the sequential proteolytic cleavage of the amyloid precursor protein (APP).[5] Beta-site APP cleaving enzyme (BACE-1) is responsible for the first step in this process, and it is considered an attractive target for disease modification.[6−9] Several highly potent BACE-1 inhibitors have been developed by various groups. However, many of these compounds have suffered from undesirable pharmacological properties, e.g., low brain exposure.[10−12]

Several lead generation screening campaigns have been performed to find starting points for BACE-1 inhibitors.,[10,13−17] Many of the identified hits have been based on amidine or guanidine core structures, containing a basic center and two hydrogen bond donors that facilitate high target affinity (Figure 1).[15] However, for some core types there have been reported difficulties to achieve high brain exposure in animals in vivo.[15,18,19] In addition, the basic center surrounded by aromatic and quite lipophilic substituents is prone to induce a risk for hERG activity.[17,20−22] Accordingly, the core structure plays a crucial role for the profile of the final test compounds.

Computer-aided design of BACE inhibitors have previously been reported.[23−25] In this work, we set out to extensively explore several de novo designed cores with the aim to allow for test compounds with improved properties. A lead generation workflow was set up for iterative creation and prioritization of core structures with promising in silico profiles regarding all three of our chosen design parameters: BACE-1 activity, permeability, and low hERG activity (Scheme 1). Once promising new cores were identified, the R1 and R2 substituents would later be subjected to further lead optimization (Figure 1).[26]

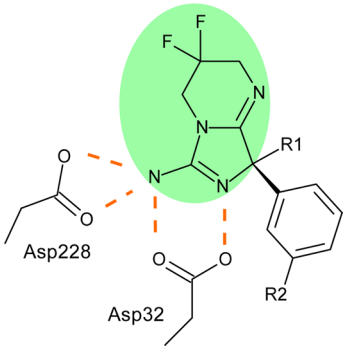
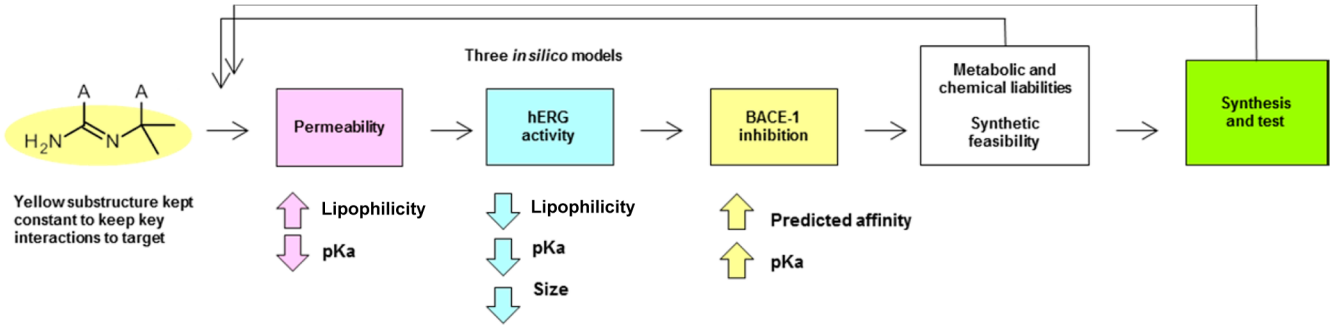


Figure 1. Example of a BACE-1 inhibitor, with the key ionic and hydrogen bonding interactions to the catalytic aspartates, as confirmed by crystal structures (orange dotted lines, distances from 2.6 to 2.8 Å). The core is drawn on green background. The favored meta-position of R2, extending into the S3 pocket of the active site, has previously been reported.[15]

Prior to setting up the lead generation workflow, Free−Wilson analyses[27] were performed on selected in-house compounds with the amidine or guanidine core types. The

Received: January 29, 2014
Accepted: February 3, 2014
Published: February 3, 2014

Scheme 1. Overview of the Multiparameter Lead Generation Workflow for Iteratively Prioritizing and Designing Amidine or Guanidine Core Structures As Starting Points for BACE-1 Inhibitors



structural elements of the core and R1 and R2 substituents were separated and an additive SAR was shown for target activity, pK_a, and hERG activity (Table S1, Supporting Information). The analyses also generated quantitative values for each core's contribution to the in vitro results of the test compounds, within the defined error margins (Table S2, Supporting Information). This information was used to facilitate core-to-core comparisons. In addition, the physicochemical properties of the cores could be compared to target activity, permeability, and hERG activity. Analyses of these comparisons were then used for setting up the predictive in silico models described below.

The guiding design parameters (colored arrows) were chosen based on hypotheses drawn from analyses of how these physicochemical properties of the cores correlate to in vitro data for previous series and reference compounds (Supporting Information). Arrows indicate parameter direction with the aim to increase BACE-1 cell activity and permeability and decrease hERG activity. Interestingly, the arrows for pK_a and lipophilicity are pointing in opposite directions, posing a challenge to achieve the optimal physicochemical profile. Before synthesis, the de novo designed cores were analyzed in a manual assessment step by experts regarding synthetic feasibility and metabolic stability. Results from synthesized core compounds were fed back into the design loop to enable learnings and continuous refinements of the predictive models.

In order to create an initial number of de novo designed core structures, the substructure in Scheme 1 was used as a basis for idea generation and design. By collecting ideas from medicinal chemistry colleagues, both with and without previous experience with BACE-1, we aimed to increase the diversity of the initial library. As a result, several hundred de novo designed cores were generated, ranging from 4- to 7-membered cyclic amidines and guanidines. In total, the initial de novo designs, together with additional ideas generated by iterations, added up to 785 structures.

Three predictive in silico tools were applied to aid de novo design and to facilitate fast progression and iterative learning (Scheme 1). The permeability model was based on the hypothesis that both pK_a and lipophilicity of the cores need to be in the right range.[28,29] Thus, cores with calculated solvation energy (describing lipophilicity) above −13 kcal/mol and a predicted pK_a below 9.5 were hypothesized to have an increased probability of high brain exposure. Further details on how these limits were chosen and how the calculations were performed are described in the Supporting Information.

The second model agreed with previously reported guidelines to lower hERG activity, i.e., to decrease pK_a, lipophilicity, and size.[30,31] Lipophilicity was again described by the solvation energy parameter, and an upper limit was hypothetically set to −10 kcal/mol. This boundary allowed for a span in solvation energy that we assumed would be consistent with both permeable and less hERG active cores. Further details on the size limit and a QSAR model for hERG activity are found in the Supporting Information.

The third predictive model estimated the relative binding affinity of the cores to the catalytic aspartates.[2] This method was developed in parallel with this work, to guide prediction of BACE-1 activity. In addition, it has been reported that pK_a should be higher than 4.5 for a core to be protonated in the cellular compartments where BACE-1 is active,[7] and a further increase of pK_a has been positively correlated with an increased target inhibition in cells, as compared to the enzymatic assay.[15,22,32,33] Here, the cell assay was regarded more relevant to the in vivo situation.

In summary, all in silico models were based on quantum mechanical calculations on the core alone, with R1 as methyl and the phenyl replaced by methyl (Figure 1). This computationally demanding method was chosen intentionally since we wanted the prediction methods to be independent of training and suitable for describing the electronically diverse cores. Approximately 70% of the suggested cores were down-prioritized by the predictive models for permeability, hERG, and activity (Figure 2).

After in silico assisted prioritization, the cores were subjected to an additional manual evaluation with respect to metabolic liabilities. The objective in this step was to identify suggested cores with structural elements that could constitute an increased risk for side-effects. For instance, cores containing isolated double bonds were discarded because of the increased metabolic risk of forming epoxides and reactive metabolites. Reactive groups such as alkyl halides and Michael acceptors were down-prioritized, as well as assumed metabolically instable groups. Synthetic feasibility was the last step in the manual evaluation, considering availability of starting materials, precedence in the literature, and number of synthetic steps.

The most promising cores were selected for synthesis. By covering a reasonable part of the defined interesting chemical space, we hoped to find cores with the most well balanced physicochemical properties (Figure 3). During lead optimization, this balance may then be further fine-tuned: for instance, pK_a can be slightly decreased or increased by using electron withdrawing or donating R1 and R2 substituents, or by modifying the phenyl in Figure 1.[22,33,35]

All of the selected core structures were synthesized with well-characterized substituents from previous in-house series.[1] There

dx.doi.org/10.1021/ml5000433 | ACS Med. Chem. Lett. 2014, 5, 440−445

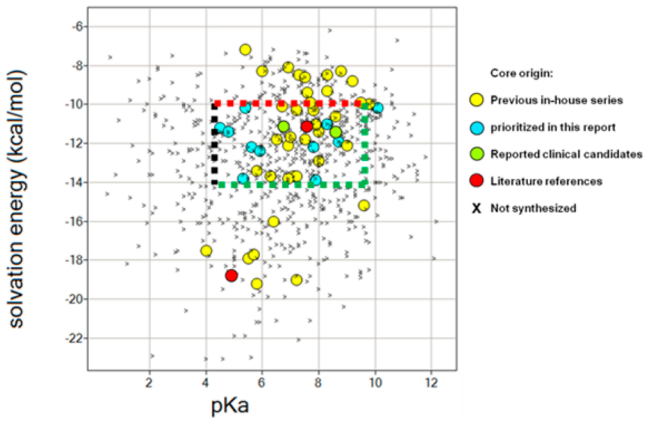


Figure 2. Defined area within the chemical space of solvation energy and pK_a was used to guide prioritization of the suggested core structures. The limits are set by the in silico models. Green lines, permeability model; red line, hERG model; black line, lower pK_a limit for cores to be protonated in the cellular compartments containing BACE-1. Structures of the prioritized cores, reported clinical candidates, and the literature references are found in Figure 3.

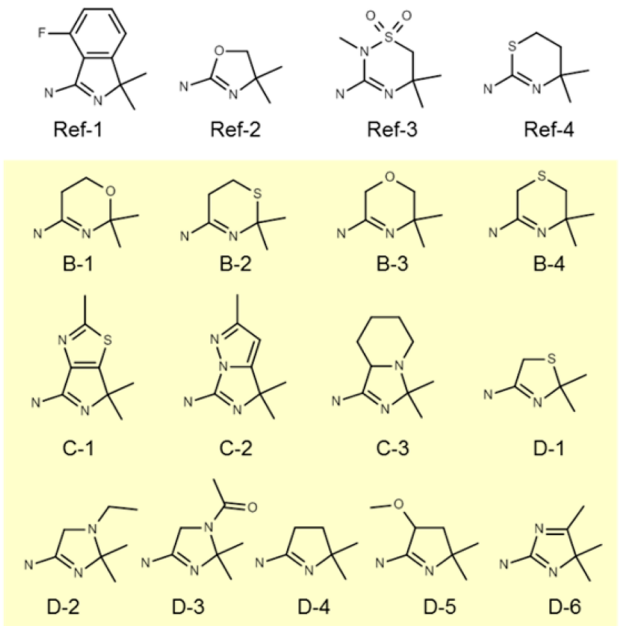


Figure 3. Overview of the prioritized and successfully synthesized cores on yellow background. The nomenclature is consistent with a previous article.[1] ref-1 and ref-4 represent known clinical candidates.[22,34] Compounds representing ref-2 and ref-3 were not synthesized and tested in this work but have been reported in the literature as having promising brain efficacy.[10,36,37] Two of the cores (D-2 and D-6) were shown to be chemically unstable and the in vitro data was not considered for those. During this work, compounds representing four of the cores (B-1, B-2, B-3, and C-1) were independently published by others.[38−40]

were four reasons for immediately converting the core fragments into full-sized test compounds: (1) With a molecular weight above 300, we wanted to avoid a seemingly high permeability only due to a low molecular weight.[28,29] (2) By testing larger molecules, with, e.g., higher target activity compared to fragments, we wanted to enter a reliable detection range in the in vitro assays. (3) We have experienced that it is often easier to isolate and purify drug-like compounds, as compared to the (usually) more water-soluble fragments. (4) As described above, the Free−Wilson analysis had shown an additive SAR for the optimization parameters investigated, and thus, decoration with common R-groups from previous series facilitated matched-pair comparisons to previous in-house series. Consequently, we assumed that only a few synthesized compounds per core would be needed for making quick assessments of whether the properties of the new cores were promising or not.

In total, 20 compounds were synthesized and tested in vitro with respect to target activity (enzymatic and cellular), permeability, hERG activity, and pK_a.[1] To analyze the properties of the new cores, and to compare them to the literature references and previous in-house series, the Free−Wilson analyses were performed again. Data generated by the first synthesized compounds were also used to refine the predictive models and to guide further de novo design of additional cores. The Supporting Information (Table S2) contains all core-related in vitro data based on these new compounds, together with the training set of cores from previous in-house series and the literature.

During the evaluation of the new cores, we experienced that this lead generation method was indeed useful for quickly finding active cores with diverse in vitro profiles. We were pleased to find that all new cores were active on target, with sometimes higher cellular potencies than the references representing two known clinical candidates (Figure 3). It was also encouraging to find several cores with even lower intrinsic hERG activity and equivalent permeability profiles as compared to these references.[1]

The physicochemical properties of the new cores showed the correlations to cellular target potency, permeability, and hERG activity as shown in Scheme 1. Thus, because of their inversely correlating relationships, it was not trivial to optimize all these properties into one single core. Still, with this method we found that it is possible to create promising cores that can be subjected to further lead optimization.

Knowledge for future permeability predictions was also generated: with permeability data on the selected cores added to the chemical space of solvation energy and pK_a, the previously defined permeability window could be revised, and instead, a triangle was proposed (Supporting Information and Figure 4). To our knowledge, the only significant outlier in our defined space for highly permeable cores was ref-3, which has been reported as having an "unexpectedly reduced susceptibility to efflux by P-gp."[37] However, in the area of reasonable pK_a and lipophilicity, there is a very fine line separating the highly and poorly permeable cores, still making it nontrivial to predict permeability correctly.

This lead generation strategy relied on three predictive models as the primary tools for aiding prioritization of several hundreds of de novo designed cores for synthesis. In the literature, it has been more common to screen in-house or commercially available libraries.[13,16,17,41−45] To illustrate the risk of missing interesting starting points when screening only available sources, substructure searches were performed for all 785 suggested cores. Using Chemistry Connect,[46] they were compared to all available compounds in commercial databases, the literature, and our internal collection. Indeed, the overlap was very small (<5%), and most matches were compounds generated by previous in-house work or found in publications. Some of the suggested virtual cores were found as substructures in our compound collection, but with the wrong substitution

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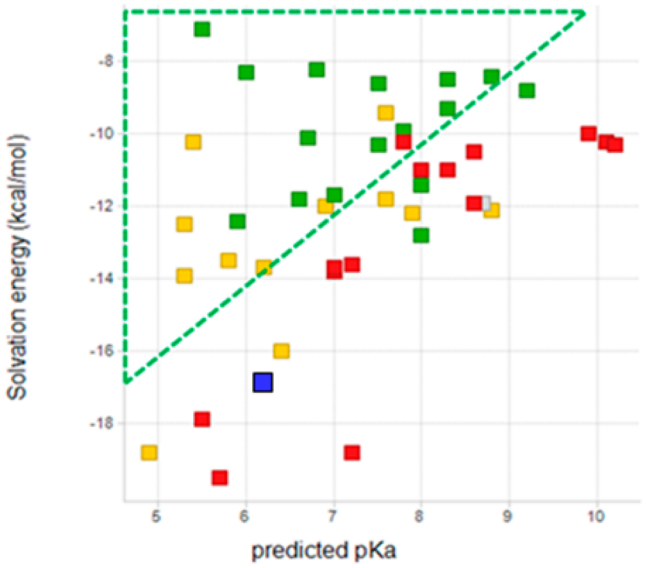

Figure 4. Within the physicochemical space of calculated solvation energy and pK_a, we propose a triangular area (green dotted line) to enrich on cores with good permeability, according to in vitro results for the test compounds. The colors green, yellow, and red indicate classification of good, medium, and poor permeability of the cores. Blue: ref-3. Data from Table S2 in the Supporting Information.

pattern to fit the active site of BACE-1, and thus, they would not have shown up as hits in an in vitro screen.

In summary, iterative de novo design assisted by multiparameter in silico predictions and subsequent core-to-core comparisons based on in vitro data allowed the identification of novel and promising starting points for optimization. Despite the need for demanding synthesis, we believe that it is worthwhile to focus on identifying the most promising cores prior to optimizing the R-groups. In this case, we could take advantage of additive SAR, which facilitated matched-pairs comparisons. Thus, only a few synthesized compounds were required to get strong indications of how a core will affect the in vitro properties of a final compound. This lead generation strategy may be particularly useful in other drug design projects utilizing an amidine or guanidine core structure.[47] We also believe this strategy may be useful in general, as a method to guide scaffold hopping.

■ ASSOCIATED CONTENT

Supporting Information

Method descriptions of the Free–Wilson analyses, calculations of predicted pK_a and solvation energy, 2D structures of the cores together with core-related in vitro data, and analysis on how the physicochemical properties of the core structures correlate to this data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Kim Paulsen for performing the Chemistry Connect substructure searches. We also thank Jörg Holenz and Sven Hellberg for valuable comments and proofreading of the manuscript.

■ ABBREVIATIONS

AD, Alzheimer's disease; APP, amyloid precursor protein; BACE-1, beta-site APP cleaving enzyme; DFT, density functional theory; SAR, structure–activity relationship; P-gp, P-glycoprotein; hERG, human ether-à-go-go-related gene

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