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Hypothesis

A 3D-QSAR model based screen for dihydropyridine-like compound library to identify inhibitors of amyloid beta (Aβ) production

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Abstract:
Abnormal accumulation of amyloid beta peptide (Aβ) is one of the hallmarks of Alzheimer's disease progression. Practical limitations such as cost , poor hit rates and a lack of well characterized targets are a major bottle neck in the in vitro screening of a large number of chemical libraries and profiling them to identify Aβ inhibitors. We used a limited set of 1,4 dihydropyridine (DHP)-like compounds from our model set (MS) of 24 compounds which inhibit Aβ as a training set and built 3D-QSAR (Three-dimensional Quantitative Structure-Activity Relationship) models using the Phase program (SchrÖdinger, USA). We developed a 3D-QSAR model that showed the best prediction for Aβ inhibition in the test set of compounds and used this model to screen a 1,043 DHP-like small library set of (LS) compounds. We found that our model can effectively predict potent hits at a very high rate and result in significant cost savings when screening larger libraries. We describe here our in silico model building strategy, model selection parameters and the chemical features that are useful for successful screening of DHP and DHP-like chemical libraries for Aß inhibitors.

Key words: 3D-QSAR, β-amyloid, in silico screening, dihydropyridine, Alzheimer's Disease

Background:
Alzheimer's disease (AD) is an ever-increasing health concern among the aging population and is the most common form of dementia affecting more than 25 million individuals worldwide **[1].** While the cause of the disease is uncertain, there are two major neuropathological hallmarks present in the brains of AD patients at autopsy: the extracellular senile plaques and the intracellular neurofibrillary tangles **[2].** Neurofibrillary tangles contain hyperphosphorylated microtubule-associated protein tau, while senile plaques contain a core of β-amyloid (Aß) peptide. Current treatments for AD include cholinesterase inhibitors and glutamate antagonists **[3].** Although useful, these symptomatic treatments do not stop the disease process or prevent neuronal degeneration **[4].** There is an on-going need for the development of new treatments for AD. Although the central role of Aβ therapy remains to be proven in clinical trials, data over the past two decades place the accumulation of Aβ peptides and, in particular, soluble forms of these peptides as key molecules initiating the pathological cascade that eventually leads to the full pathology of AD. Consequently, significant resources have been allocated to the discovery of new pharmaceutical entities that have Aβ-lowering properties.

Drug discovery is typically a complex multi step process that involves screening of a large number of compounds for potential 'hits' in relevant assays. Using information from these hits, medicinal chemists design and synthesize compounds to identify novel molecules or optimize compounds into suitable leads that can be tested in preclinical experiments. Available chemical libraries are expanding at a rapid pace due to combinatorial chemistry thus allowing exploration for inhibitors in new chemical space. However, the cost of identifying hits from assay screens of vast libraries can be cost prohibitive. One way to reduce cost is to limit screening to a focused library of compounds that represent the chemical space of interest constituted by related chemotypes. With advances in combinatorial synthesis technology, several thousand compounds can be rapidly synthesized thus expanding the relevant chemical space **[5].** Hence, there is a need for predictive models like QSAR (Quantitative Structure Activity Relationship) that will prioritize compounds for screening, aid rational synthesis and facilitate lead identification **[6].** A QSAR model is useful in relating biological activity to physico-chemical and structural descriptors of compounds. By applying QSAR techniques, lead compounds have previously been identified for a range of biological targets **[7].** A 3D-QSAR model is built using the alignment of three dimensional conformers of active compounds and can be subsequently used to score a candidate compound on the basis of a fitting function that evaluates the alignment of three dimensional chemical features to the model **[8-10].** A pharmacophore is constituted by common chemical features (such as hydrogen donors, hydrogen acceptors, hydrophobic groups, charged groups and aromatic rings) that are distributed spatially to interact with the biological target and exert activity **[11].** Development of 3D pharmacophore models based on the biological activity of compounds enables ligand-based drug design that guides experimental chemical synthesis of compounds with higher potency even when the 3D structure of the biological target is unknown **[12].**

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During the course of the examination of risk factors for AD, such as hypertension, it has become clear that certain anti-hypertensive compounds may be protective, not just against stroke-related dementia, but also independently against AD. For instance, in the Syst-Eur trial, which involved active treatment with the dihydropyridine (DHP) calcium channel blocker (CCB) nitrendipine in over 2,400 patients, there was a 55% reduction in the incidence of AD **[13].** Nitrendipine and nilvadipine are closely related DHPs that provide clinical protective signals against AD whereas amlodipine and nifedipine do not confer such protection. Clearly then, the AD protection afforded by some of the members of the DHP class of antihypertensive drugs appear to be unique to a subgroup of the class and is not related to the antihypertensive activity of the compounds. Since Aβ is implicated in Alzheimer's disease, we set out to explore whether DHP-like compounds can inhibit Aβ production in an engineered mammalian cell that over expresses it. Although some of the DHPs inhibited Aβ production, the exact target is unknown, therefore, we used a ligand based approach to build a 3D QSAR model using few model compounds. Using a DHP-like model set (MS) of compounds we built a QSAR model with Phase software (Schrodinger, USA) to predict the activity of compounds in a small library set (LS) **(Figure 1).** We describe here our 3D-QSAR model building approach, the application of the model to screen for hits in a DHP-like compound library and its ability to identify potent new hits in a compound library of DHP like structures.

Methodology :

Measurement of Aß inhibition

A cell based assay was used for screening of the DHP and DHP-like compounds using Chinese Hamster Ovary cells stably transfected with wild-type APP751 (7W cells) overproducing human Aβ **[14]** that we previously used to identify Aβ lowering compounds **[15].** Briefly, 7W cells were grown in DMEM (ATCC, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 1× Penicillin–Streptomycin Fungizone mixture (Cambrex, Rockland,ME, USA) and 0.3% Geneticin (Invitrogen, Carlsbad, CA, USA). Cells were plated on 96 well-culture plates at a density of 5×104 cells per well in 200 µL of culture medium. Culture medium was replaced in each well with 200 µL of fresh culture medium containing the vehicle (DMSO) or 5 µM of the compound, 24 hours after plating. On each 96 well-plate, 8 wells were used as control to determine the basal Aβ production and up to 80 compounds per plate were tested. Following 24 hours of incubation with the compounds, Aβ1-40 and Aβ1-42 levels were evaluated using commercially available Aβ ELISAs (EMD Biosciences Inc., CA, USA). Toxicity of the compounds was evaluated by monitoring the release of lactate-dehydrogenase release in the culture medium using a cytotoxicity detection kit (Roche, Inc.). Compounds showing cellular toxicity were discarded from further analysis.

Calculation of IC50 for selected compounds

Twenty four seed compounds that contain a DHP core (4-phenyl-1,4-dihydropyridine [Smiles: C1(C=CNC=C1)C2=CC=CC=C2]) were tested in quadruplicate at 0.1; 0.5; 2.5; 5;10 and 50 µM in 7W cells for their effects on Aβ. These seed compounds were selected based on ready commercial availability during our initial screen and have different potency towards Aβ inhibition. The half maximal inhibitory concentration (IC50) of the compounds for Aβ was calculated with the software Graphpad Prism V5 for Windows.

Development of a 3D-QSAR model

Our model set (MS) contained twenty-one "seed" compounds and were determined to have IC50s for Aβ40 lowering ranging from 200nM to 20 µM. All IC50s were converted to a logarithmic scale using the formula,

pIC50 = -log10IC50. We used the MS compounds to build and validate a suitable QSAR model using Phase software available in the Schrordinger Package (release 2009). Phase uses a multi-step approach to model build. First, 2D chemical formulas were processed using LigPrep, to convert 2D into 3D conformers. Each of these conformers were energy minimized using MacroModel by applying an OPLS 2005 force field. The library of energy minimized conformers was used as input to the Phase program. We randomly selected 70% (17) of the MS compounds as a training set to build the model while the remaining 30% (7 compounds) served as a test set to evaluate the robustness of the model. An extensive list of atomic groups and bond patterns are available within Phase to map pharamacophore features such as Hydrogen Acceptor (A), Hydrogen Donor (D), Hydrophobic (H), Charged groups (N or P) and Aromatic ring (R). Features were mapped for each of conformer using the default list of SMARTS pattern to identify spatial distribution of pharmacophore features in different conformers. A group of common physico-chemical features aligned in 3D space forms the basis for site point and a set of site points forms the basis for a pharamacophore. In the Phase program a site point is identified as one of the several possible conserved chemical features and a specific combination of site points (or features) form hypotheses variants to define a pharmacophore **[16].** Thus a pharmacophore consists of a set of pharmacophore site points found common among active ligands. In order to find a common pharmacophore, a tree-based partitioning technique is applied to group intersite distances of all active compounds in a 16Å box. The minimum distance between two sites was kept at 2 Å to reject closely positioned pairs of features. The maximum depth of the tree was set to 4 branches. For this study we identified a five point pharmacophore, AADHR **(Figure 2)** consisting of two distinct hydrogen acceptor sites (A), one hydrogen donor site (D), one hydrophobic site (H) and one aromatic group feature (R). The geometrical features of the hypotheses can be extracted as a set of intersite distances and angles connecting every three sites. For example, the intersite distance between A1 and A4 is 7.158 Å, between A2 and D1 is 4.938 and D1 and A1 was 4.839 Å. The angle between D1, A2 and A1 is 42.4°. The feature AADHR had the highest specificity and survival score among all competing hypotheses. The AADHR was used to build a QSAR model derived from a regular grid of cubic volume elements that span the space occupied by the training set of ligands. The pharmacophore features that were present in the 3D cubic grid were scored for all compounds. The entire workflow is shown in **Figure 1.**

Focused Chemical Library for DHPs

We used the dihydropyridine core ring as a query in the TimTec library (http://www.timtec.net) to obtain 95% substructure similarity. There were a total of 1043 DHP and DHP like compounds that were pre-filtered for drug-like properties. This is referred to as the library set (LS) of focused DHP and DHP-like compounds.

Prediction of hits using the 3D-QSAR model

We performed an in vitro screening of the 1043 DHPs and DHP-like compounds in our cell based assay to measure the inhibition of Aß. We applied our 3D-QSAR model based on the AADHR pharmacophore to predict compound activity and rank them based on their predicted potency. The model was built using a maximum of three partial-least squares factors. The model's robustness was tested for its ability to predict both the training set and the test set. The R2 value of training pIC50 prediction was 0.81 (significance $p<0.05$) and the model had a large F-score of 18.6 (p-value $5.45x10^{-5}$) indicating a high confidence in the model **(Figure 3).** The test compound activities were predicted with an R2 of 0.56. We used this 3D-QSAR model to predict and rank the 1043 LS compounds. We further compared the rank of predicted potency using our model with experimentally observed inhibition of the LS compounds.

Activity based classification of Compounds:

We empirically classified LS compounds based on the % inhibition as Strong (SH), Medium (MH) and Weak hits (WH). There was a strong correlation between Aβ 40 and Aβ 42. Since the dynamic range of Aβ 40 inhibition due to abundance is greater than Aβ 42, we classified the compounds based on Aβ 40 inhibition. SH compounds are those that inhibited 90% Aβ 40 production at 5 µM. MH compounds inhibited Aβ 40 production between 60% and 89% at 5 µM, while WH compounds inhibited Aβ 40 production between 40% and 59% at 5 µM. Any compound lower than 40% was considered inactive for the purpose of this study. There were a total of 56 SH, 146 MH, 173 WH and 668 inactive compounds in the LS.

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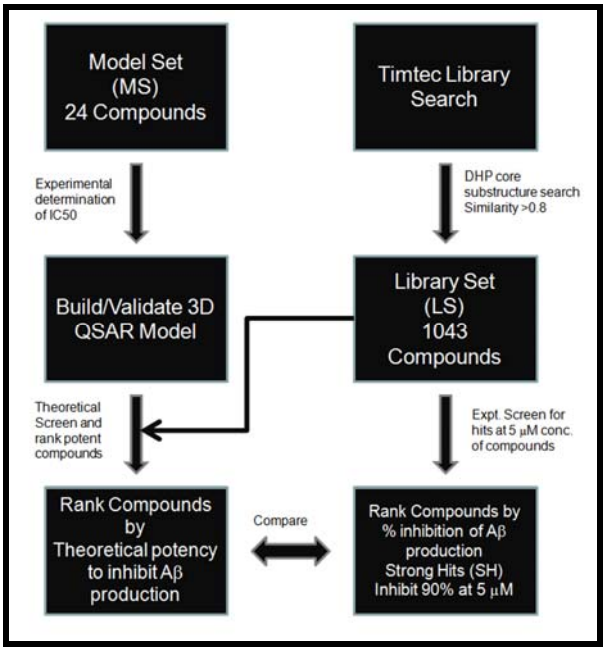

Figure 1: Workflow followed in this study.

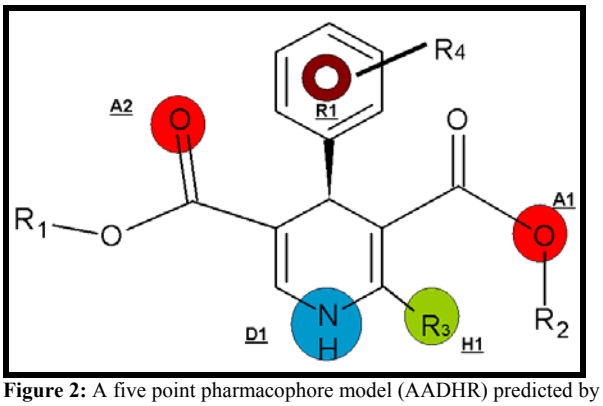

Figure 2: A five point pharmacophore model (AADHR) predicted by Phase for DHP and selected for our study. The 3D-geometric features associated with the model are detailed in Table 1. Underlined alphabets identify different features like Hydrogen Acceptor (A1,A2), Hydrogen Donor (D1), Aromatic Ring (R1) and hydrophobic group (H1).

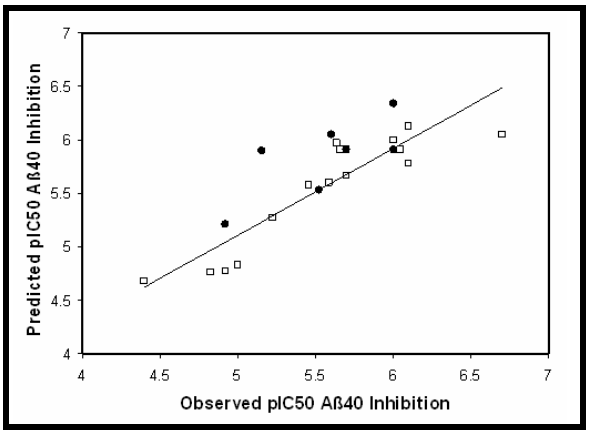

Figure 3: Predicted vs Observed pIC50 of 24 DHP like compounds with test sets shown in filled circles and training set in open squares. The correlation coefficient of predicted vs observed pIC50 is 0.9 ($p<0.05$) for the training set and for test set it is 0.75 ($p<0.05$).

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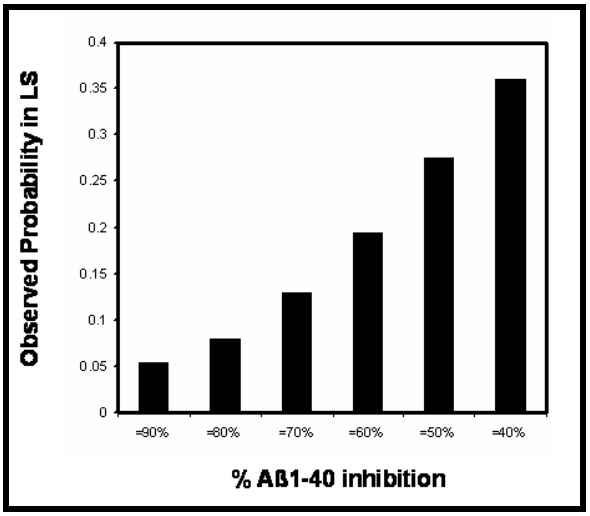

Figure 4: Distribution of active compounds in the LS based on % inhibition of Aβ40 production at 5 μM.

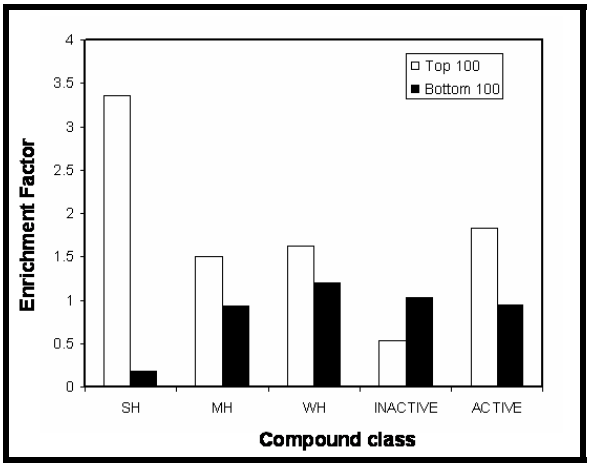

Figure 5 : Enrichment of different classes of active compounds in the top 100 predicted compounds. SH compounds are highly enriched (factor of 3.35) in the top 100 while inactive compounds are depleted. Overall active compounds (sum of compounds in SH, MH, WH) were enriched by a factor of 2 in the top 100 predicted by our QSAR model.

Results & Discussion:

Three dimensional QSAR model

We developed a ligand-based 3D QSAR model for predicting Aβ lowering activity of DHP-like compounds using the Phase program (Schrödinger Modeling Package) **[16].** Phase uses a conformational sampling and a scoring technique to match physico-chemical features of the atoms of a compound that may be critical for its biological activity. The 3D QSAR model is based on the spatial alignment of the chemical features of a training list of compounds using a virtual 3D lattice. To this 3D lattice, a weighted list of physico-chemical features (hydrophobicity, partial-charges, hydrogen donor, hydrogen acceptor, geometry) that are common among active compounds was calculated and the distribution of the features in a 3D space was extracted as vectors. We selected a model set of 24 DHP and DHP-like compounds that display a range of potencies for Aβ inhibition and calculated their corresponding pIC50 (-logIC50). We randomly selected 17 of those compounds as a training set to build the QSAR models. The best model was selected based on its ability to predict the Aβ lowering activity of the training set (correlation coefficient of 0.9). This model was applied to predict the theoretical potency or pIC50 of the remaining seven compounds and the model predicted the pIC50 correlation coefficient of 0.75 **(Figure 2).** The chemical features of the model consists of two hydrogen acceptor vector sites (A1 & A2), one hydrogen donor vector (D1), one aromatic ring vector (R1) and one hydrophobic group (H1). **Table 1 (see supplementary material)** provides a list of distance and angle separations that are characteristics of spatial chemical feature distribution in the 3D grid.

Distribution of hits in DHP-like chemical library

In our in vitro screen of LS compounds at 5 μM, there were 56 SH compounds that inhibited Aß by more than 90% (random probability of finding, Pr = 0.054), 146 compounds had medium potency or MH (Pr = 0.14), 173 compounds were WH (Pr=0.17) and 668 compounds were classified as inactive (Pr=0.64). A model with true predictive power must be able to identify potent compounds in the top ranking compounds based predicted potency. Enrichment of potent hits among top ranked compounds based on predicted potency will significantly reduce cost and save time in screening large chemical libraries. **Figure 4** shows the distribution of inhibitors based on the % inhibition of Aβ 40 production at 5 μM in a cell based assay for the chemical library of 1043 compounds.

Predicting DHP-like hits using in silico screen

We applied our 3D-QSAR model to predict the Aβ lowering activity of the compounds in a focused DHP library and selected the top and bottom 100

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compounds based on their predicted potency. Among the 100 bottom compounds selected by the model, 99% showed no Aβ lowering activity and only one compound displayed marginal Aβ lowering activity (20% inhibition at 5 μM). Within the top 100 compounds, the model identified over 66% of active compounds, among them 18 were the most potent Aβ lowering SH compounds in the library. The probability of finding SH compounds in the top 100 is three times greater using the QSAR model compared to finding them by chance. In the bottom 100 compounds we found just 1 of the SH compounds and 66% of the inactive compounds, implying our method selectively depleted active compounds in the bottom ranking **(Figure 5)**. Thus our prediction model enriches potent compounds in the top 10% ranking based on predicted potency while depleting potent compounds in the bottom 10%. By screening top 30% of compounds as predicted by our model we demonstrate that one must be able identify 73% of all potent compounds that inhibit Aβ 40 production at least 90% at 5 μM.

Our data show, despite limited information that it is possible to build a working 3D QSAR model to predict the Aβ lowering activity of DHP compounds and hence perform an in silico screening of a focused library. Although, a model built with a large training set of compounds may refine predictive power, even a limited number of compounds fitted in the model is sufficient to allow an enrichment of a focused library for active compounds.

3D-QSAR model prediction performance

Screening for hits in large chemical libraries to identify inhibitors of Aβ production is an expensive proposition. Based on in house experiments (unpublished) that demonstrated DHPs were able to ameliorate AD pathology, we set out to screen a small focused library of DHP like molecules and determined their IC50s for Aβ production. Currently there are more than 35,000 compounds with DHP like cores available from public databases. Screening such vast libraries in vitro is an expensive process due to the cost of the ELISA used in quantitating Aß. We set out to build a 3D-QSAR model using Phase with a limited set of compounds for which the IC50s for inhibition of Aβ production were known from our in vitro cell based assay. The best 3D-QSAR model was able to predict the test set with a correlation coefficient of 0.7. We applied this model to predict the inhibitory potency of an intermediate sized focused DHP library and to test the feasibility of applying an in silico screen to prioritize compounds for in vitro screening. Using an iterative screening and model building process the performance of the model can be improved further. To accomplish this, we intend to apply 3D-QSAR modeling to screen a larger library of DHP and DHP-like compounds from several other commercial and public sources.

Conclusion:

We demonstrate here that a simple 3D-QSAR model is able to enrich for biologically potent compounds in the top 10% arranged by predicted potency while depleting them from predicted low activity compounds. Our approach can be combined with other predictive models for ADME properties and linear 2D based models to rapidly screen large chemical libraries in order to prioritize potent compounds for further in vitro screening.

Acknowledgements

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Supplementary material:

Table 1: Geometric constrains of pharamacophore features identified in this study to predict Aβ production inhibitors using a 3D-QSAR model by Phase (Schordinger, USA). There are two Acceptor (A1, A2), one donor (D1), one aromatic (R1) and one hydrophobic site (H1).

Site1	Site2	Distance (Å)	Site1	Site2	Site3	Angle (in degrees)
A2	A1	7.158	A1	A2	D1	42.4
A2	D1	4.938	A1	A2	H1	23.7
A2	H1	6.594	A1	A2	R1	39
A2	R1	4.797	D1	A2	H1	19.1
A1	D1	4.839	D1	A2	R1	78.9
A1	H1	2.876	H1	A2	R1	61.8
A1	R1	4.568	A2	A1	D1	43.5
D1	H1	2.513	A2	A1	H1	67.1
D1	R1	6.185	A2	A1	R1	41.3
H1	R1	6.046	D1	A1	H1	24.3
			D1	A1	R1	82.2
			H1	A1	R1	106.4
			A2	D1	A1	94.1
			A2	D1	H1	121
			A2	D1	R1	49.6
			A1	D1	H1	28.1
			A1	D1	R1	47
			H1	D1	R1	75.1
			A2	H1	A1	89.2
			A2	H1	D1	40
			A2	H1	R1	44.3
			A1	H1	D1	127.7
			A1	H1	R1	46.4
			D1	H1	R1	81.3
			A2	R1	A1	99.7
			A2	R1	D1	51.6
			A2	R1	H1	73.9
			A1	R1	D1	50.8
			A1	R1	H1	27.1
			D1	R1	H1	23.7