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Discovery of novel mGluR1 antagonists: A multistep virtual screening approach based on an SVM model and a pharmacophore hypothesis significantly increases the hit rate and enrichment factor

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ABSTRACT

Development of glutamate non-competitive antagonists of mGluR1 (Metabotropic glutamate receptor subtype 1) has increasingly attracted much attention in recent years due to their potential therapeutic application for various nervous disorders. Since there is no crystal structure reported for mGluR1, ligand-based virtual screening (VS) methods, typically pharmacophore-based VS (PB-VS), are often used for the discovery of mGluR1 antagonists. Nevertheless, PB-VS usually suffers a lower hit rate and enrichment factor. In this investigation, we established a multistep ligand-based VS approach that is based on a support vector machine (SVM) classification model and a pharmacophore model. Performance evaluation of these methods in virtual screening against a large independent test set, M-MDDR, show that the multistep VS approach significantly increases the hit rate and enrichment factor compared with the individual SB-VS and PB-VS methods. The multistep VS approach was then used to screen several large chemical libraries including PubChem, Specs, and Enamine. Finally a total of 20 compounds were selected from the top ranking compounds, and shifted to the subsequent in vitro and in vivo studies, which results will be reported in the near future.

Metabotropic glutamate receptors (mGluRs) are members of the family C of G protein-coupled receptors (GPCRs).[1] Many studies have revealed that the mGluRs play important roles in the central and peripheral nervous system. For instance, they are involved in learning, memory, anxiety, and the perception of pain.[2,3] Currently eight subtypes of mGluRs have been cloned, which are divided into three sub-groups according to their sequence homology, pharmacology, and signal transduction mechanisms: group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4, mGluR7, and mGluR8).[4] Among these mGluRs, of special interest is the mGluR1 in group I. mGluR1 is widely distributed in the central nervous system (CNS), which modulates synaptic transmission, neuronal excitability, and brain plasticity.[5] Numerous studies have indicated that over-expression or excessive activation of mGluR1 has been implicated in a number of central nervous system disorders including pain,[6] anxiety,[7] depression,[8] and Parkinson's disease.[9] Thus antagonists of mGluR1 have been thought as potential drugs for the treatment of these diseases.

Early mGluR1 antagonists are mainly glutamate competitive antagonists, which bind to the extracellular domain (ECD) of mGluR1, the natural ligand glutamate binding site.[10] However, these glutamate competitive antagonists suffer many deficiencies, in particular low selectivity, which might lead to unexpected side effects, hence hampering them to become to drugs.[10] Thus, the focus of pharmaceutical industry and academic institutes has transferred to non-competitive antagonists that bind to the allosteric site in the seven-transmembrane domain (7TM).[11] The non-competitive antagonists are considered more selective than the competitive ones, which makes them more attractive therapeutic agents for treatment of human nervous disorders.[10] Despite many attempts in the past years, there is no non-competitive antagonist in clinical studies so far. Therefore, discovering more potent non-competitive antagonists of mGluR1 are still needed and important, which could provide more lead candidates for drug development.

Historically, the discovery of mGluR1 antagonists (hereafter antagonists are referred to non-competitive antagonists unless indicated explicitly) is mainly based on high-throughput screening (HTS) approaches, which usually suffer a high cost and a low hit rate.[12] An alternative approach is in silico virtual screening (VS), which provides an economic and rapid strategy for lead discovery.

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Commonly used VS methods include structure-based methods, such as molecular docking, and ligand-based approaches, for example pharmacophore-based VS. Nevertheless, structure-based methods are not suitable for the case of mGluR1 since for which there is no crystal structure reported at present; not just mGluR1, there is no crystal structure published yet for all the family C GPCRs. In addition, the sequence identity of mGluR1 with other GPCRs whose crystal structures are known is very low (less than 5%), which limits the application of homology modeling. Thus, the ligand-based methods are the only option for the VS of mGluR1 antagonists. The widely used ligand-based method is the pharmacophore-based VS (PB-VS).[13,14] Although PB-VS is one of the best VS methods, it still bears some inherent limitations including low speed, high false positive and low enrichment factor particularly in screening large databases.[15] Lately emerging support vector machine (SVM)-based VS (SB-VS)[16,17] seems superior to the PB-VS in some aspects, particularly the screening speed. However, the SB-VS still suffers a low hit rate and a high false positive rate, which might originate from the lack of consideration of the information regarding the three dimensional (3-D) pharmacophore features of small molecules.[15] A combination of these methods is expected to be able to mutually compensate for these limitations and capitalize on their mutual strengths.

The goal of this investigation is to establish a multistep VS approach that combines the SB-VS and PB-VS for discovering more potent antagonists of mGluR1. We shall first build an SVM-based VS model and a pharmacophore-based VS model of mGluR1 antagonists. The performance of these two models as well as their combination in the virtual screening will be evaluated, followed by applying the optimal VS model to screen several large chemical libraries, including PubChem (18,831,686 compounds), Specs (202,408 compounds), and Enamine (980,000 compounds) for identifying novel mGluR1 antagonists. Finally some of the retrieved hit compounds will be selected and shifted to the subsequent in vitro and in vivo studies.

Establishment and validation of the SVM classification model of mGluR1 antagonists and non-antagonists. SVM is actually a classifier with an optimal separating hyperplane that separate two classes in the feature space.[18] Given a set of training compounds, each marked as one of two categories (mGluR1 antagonist or non-antagonist), an SVM training algorithm builds a model that predicts whether a new compound falls into one category or the other. Here a total of 336 compounds including 303 antagonists (positives, IC_{50} or $K_i \leqslant 10\ \mu M$) and 33 non-antagonists (negatives, $IC_{50} > 10\ \mu M$) were firstly collected from literature.[14,19–28] All of these compounds were randomly divided into three groups (see Table S1–S3 in Supplementary data). The first group contains 180 positives. These compounds together with 15,288 putative negatives were used for the SVM model training; the putative negatives were obtained from 18.83 M PubChem compounds by using a method suggested by Chen and co-workers.[16] The second group comprising 60 positives and 33 negatives was adopted for the validation of SVM model. The remaining 63 positive compounds together with the 68,106 decoys[29] (the selection of decoys see Supplementary data) from MDDR (MDL Drug Data Report) library were used for the evaluation of the overall performances of SB-VS, PB-VS and their combination.

In the SVM modeling, 252 molecular descriptors, which cover various molecule properties such as geometrical, topological, electronic properties, were calculated for each of the training set compounds by using Discovery Studio (DS) 2.55 program package (Accelrys, San Diego, USA). The initial descriptors were preprocessed to eliminate some obvious 'bad' ones. Here, the following descriptors were removed: (1) descriptors with too many zero values, (2) descriptors with very small standard deviation values (<0.5%), (3) descriptors which are highly correlated with others (correlation coefficients >95%). After this preprocessing, a total of 84 molecular descriptors remained. Since different ranges of descriptor values would influence the quality of SVM classification model, all the descriptor values were scaled to a range from −1 to +1. After that, the GA-CG method proposed by us[30] was further used for descriptor (feature) selection and parameter optimization; the GA-CG method is an integrated scheme for feature selection and parameter setting in SVM modeling, in which a genetic algorithm (GA) is used for the feature selection and the conjugate gradient (CG) method for the parameter optimization. Finally, 40 molecular descriptors were chosen for training the SVM model, which can be roughly grouped into the following seven categories: constitutional descriptor (13), estate keys (15), lipophilicity descriptor (1), solubility descriptor (1), hydrogen-bonding descriptor (1), surface area and volume descriptor (1), and topological descriptor (8) (see Table 1; an interpretation for each of the selected descriptors is given in Table S4 in Supplementary data).

The generated SVM model was firstly validated by 5-fold cross validation. The validation results are summarized in Table 2. From Table 2, we can see that 158 of the 180 known mGluR1 antagonists were correctly predicted (TP, Table 2). The accuracy for the prediction of mGluR1 antagonists (SE, Table 2) is 87.78%. In the 15,288 mGluR1 putative non-antagonists, 15,264 were properly predicted (TN, Table 2). The accuracy for the prediction of mGluR1 non-antagonists (SP, Table 2) is 99.84%. The overall prediction accuracy (Q) is 99.70%. These results indicate that the generated SVM model is quite good for the prediction of training set agents.

To further assess the prediction ability of the established SVM model to external compounds outside of the training set, we established an independent test set that comprises 60 known mGluR1 antagonists and 33 non-antagonists. The prediction results are also presented in Table 2. Obviously, the established SVM model has considerably good prediction ability to the external compounds outside of the training set, implying a potential applicability of this SVM model in the virtual screening.

Table 1
Molecular descriptors selected by the GA-CG algorithm for the generation of SVM classification model of mGluR1 antagonists and non-antagonists

Descriptor class	Number of descriptors	Descriptors
Constitutional descriptors	13	C_Count, N_Count, O_Count, S_Count, Num_NegeativeAtoms, Num_RotatableBonds, Num_AromaticBonds, Num_Fragments, Num_StereoBonds, Num_AtomClasses, HBA_Count, HBD_Count, NPlusO_Count
Estate keys	15	ES_Sum_ssCH2, ES_Sum_tsC, ES_Sum_dssC, ES_Sum_ssssC, ES_Sum_sNH2, ES_Sum_aaN, ES_Sum_sssN, ES_Sum_sOH, ES_Sum_ssO, ES_Count_ssCH2, ES_Count_sssCH, ES_Count_aaaC, ES_Count_sssN, ES_Count_ddsN, ES_Count_ssS
Lipophilicity descriptor	1	*A* Log *P*
Solubility descriptor	1	Molecular_Solubility
Hydrogen-bonding descriptors	1	Num_H_Acceptors
Surface area and volume descriptor	1	Molecular_PolarSurfaceArea
Topological descriptor	8	BIC, CIC, IC, CHI_3_C, CHI_V_3_P, JX, Kappa_2, Kappa_3

Table 2
Validation results of the SVM model by a 5-fold cross validation method and an independent test set method

Test method	Number of compounds			Positive			Negative			Q[a] (%)
	Total	Positive	Negative	TP[a]	FN[a]	SE[a] (%)	TN[a]	FP[a]	SP[a] (%)	
5-Fold cross validation	15468	180	15288	158	22	87.78	15264	24	99.84	99.70
Independent test set	93	60	33	51	9	85	28	5	84.8	85.00

[a] TP, true positive; TN, true negative; FP, false positive; FN, false negative; SE(%): sensitivity, SE = TP/(TP + FN); SP(%): specificity, SP = TN/(TN + FP); Q(%): overall accuracy, Q = (TP + TN)/(TP + TN + FP + FN).

Pharmacophore modeling of mGluR1 antagonists and model validation. A pharmacophore model is an ensemble of essential chemical features necessary to ensure the optimal superamolecular interactions with a specific biological target and to trigger its biological response.[15] The purpose of pharmacophore modeling is to extract these essential chemical features from a set of known ligands, called training set. In this investigation, 10 mGluR1 antagonists (compounds **1–10**, see Fig. 1) were selected to form the training set; these compounds were chosen since they are the most active compounds and have different scaffolds. In addition, four inactive compounds[22,28] (compounds **11–14**, see Fig. 1) were also included which purpose is for seeking for the possible excluded volumes that dislike the occupying by the ligand atoms. The HipHop algorithm[31] implemented in DS 2.55 program package was used to generate common feature based pharmacophore models (a detailed description for the modeling and parameter setting, see Supplementary data). In the HipHop run, ten pharmacophore hypotheses were finally generated. The top ranking hypothesis Hypo1, which will be used for the subsequent virtual screening, contains two hydrogen-bond acceptors (A), one hydrophobic feature (H), and one hydrophobic aromatic feature (R), as well as five excluded volumes. The 3D spatial relationship and geometric parameters of Hypo1 are shown in Figure 2A. From Figure 2A, we can see that all of the four features of Hypo1 are almost coplanar, which is consistent with the fact that majority of the scaffolds of the known mGluR1 antagonists bear a good structural rigidity and coplanarity. Figure 2B–D depicts the alignments of Hypo1 with one active compound (compound **3**) and two inactive compounds (compounds **11** and **14**), respectively. Clearly, the active compound **3** was mapped very well with Hypo1 without any collision with the excluded volumes, which gives a fitness value (FitValue) of 3.89; a fitness value is a measure of how well the ligand fits the pharmacophore. Compound **11** was also mapped very well with Hypo1 (FitValue = 3.16). However some collisions exist between the benzyloxy of compound **11** and the excluded volumes, which could be used to interpret why compound **11** is inactive. For inactive compound **14**, although there are no collisions with the excluded volumes, the mapping of which with Hypo1 is not good. These demonstrate, at least to some extent, that Hypo1 is a good pharmacophore model that contains both the essential chemical features necessary for potent mGluR1 antagonists and forbidden regions which should be avoided to occupy by the antagonist atoms.

Then, a test set, M-CMC, which contains randomly selected 230 known mGluR1 antagonists and 3813 decoys[29] (the selection method for the decoys see Supplementary data) from Comprehensive Medicinal Chemistry (CMC) library, was used to examine whether the pharmacophore model Hypo1 has the ability to differentiate mGluR1 antagonists and non-antagonists. Each of the M-CMC compounds was mapped onto Hypo1. Of the 230 mGluR1 antagonists, 166 were found to map very well with Hypo1, indicating a yield of 72.17%. And 757 of the 3813 (19.85%) decoys were also superposed very well with Hypo1. These results indicate that Hypo1 is capable of discriminating to some extent the mGluR1 antagonists and non-antagonists. But the sole use of Hypo1 in VS may suffer a high false positive rate, which is one of the most important reasons why we adopt the multistep ligand-based virtual screening strategy here.

Evaluation of the overall performance of SB-VS, PB-VS and SB/PB-VS in virtual screening. In order to evaluate the overall performance of SB-VS, PB-VS and the multistep VS approach SB/PB-VS in virtual screening, we firstly constructed a large independent test set, called M-MDDR. The M-MDDR comprises 63 known mGluR1

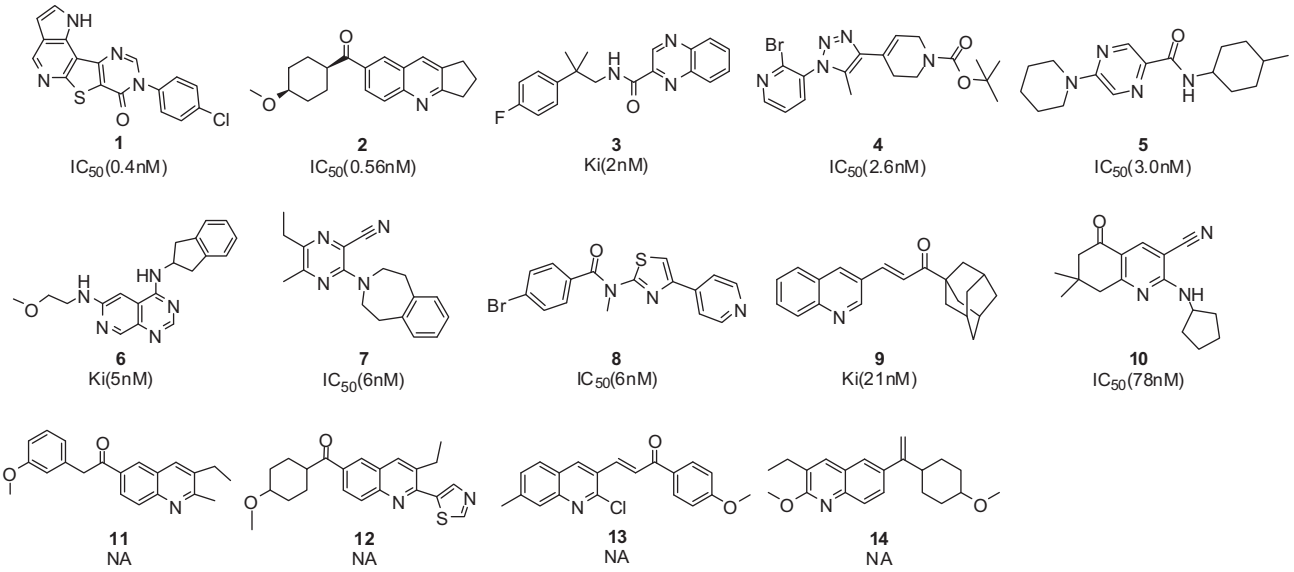

Figure 1. Chemical structures of the training set compounds for the pharmacophore modeling. NA means inactive.

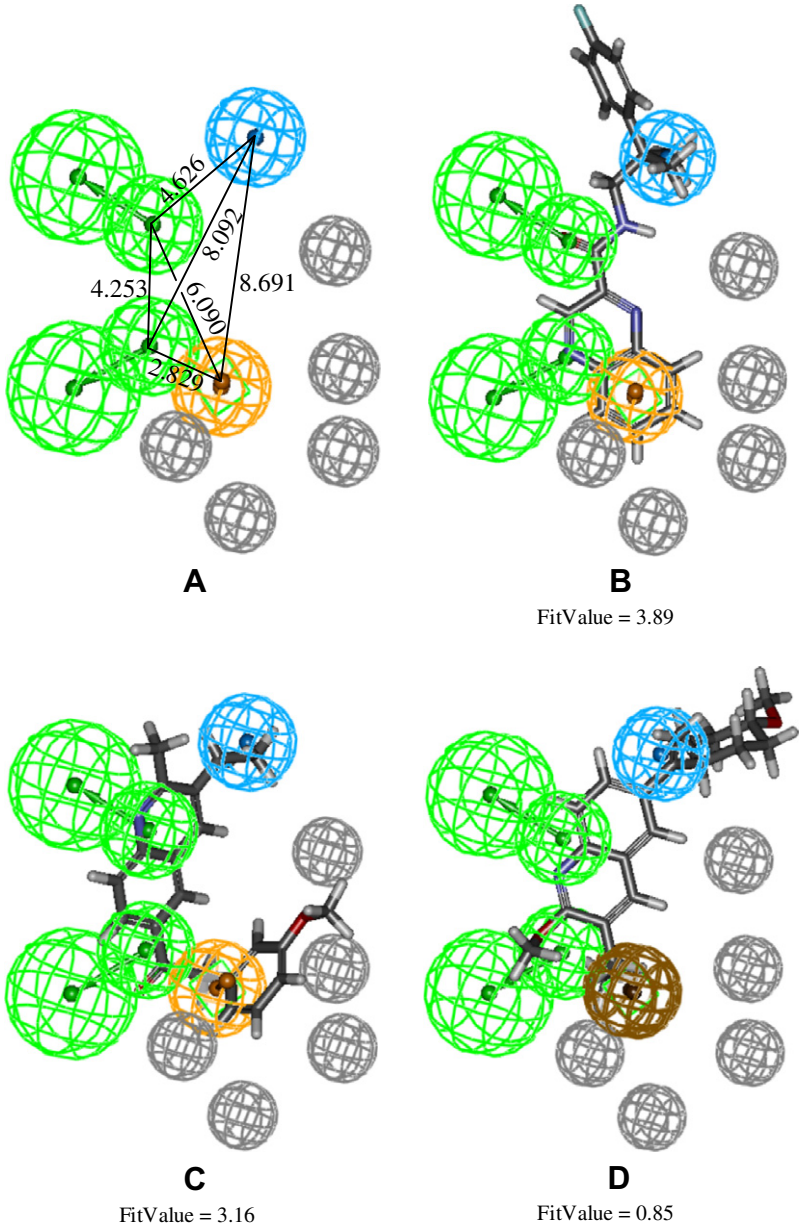

Figure 2. (A) The 3D spatial relationship and geometric parameters of the best HipHop model Hypo1. (B) Mapping of compound **3** with Hypo1. (C) Mapping of compound **11** with Hypo1. (D) Mapping of compound **14** with Hypo1. The features are color coded: green, hydrogen-bond acceptor; dark blue, hydrophobic aliphatic feature; orange, ring aromatic; grey, excluded volume; and dark orange, not mapped.

antagonists (these compounds have never been used in previous modeling and model validations), and 68,106 decoys obtained from MDDR (MDL Drug Data Report) library (the selection method of the decoys see Supplementary data). For the performance of virtual screening, the yield (percentage of predicted compounds in known antagonists), hit rate (percentage of known antagonists in predicted compounds), and enrichment factor (ratio of hit rate to the percentage of known antagonists in M-MDDR) which shows the magnitude of hit-rate improvement over random selection, were evaluated.

Firstly, SB-VS and PB-VS were individually employed to screen the M-MDDR database. The screening results are given in Table 3. From Table 3, we can see that the number of the predicted positives is 1014, and that of the hits is 54 with a yield of 85.7% (54 out of the 63 antagonists, see Table 3) for the sole use of SB-VS. The hit rate and enrichment factor are 5.33% and 57.57, respectively. The

Table 3
Comparison of the performances of SB-VS, PB-VS and SB/PB-VS in virtual screening against an independent test set, M-MDDR (63 antagonists + 68043 decoys)

Method	Predicted positive	Hits	Yield (%)	Hit rate (%)	Enrichment factor	Time cost (h)
SB-VS	1014	54	85.7	5.33	57.57	0.017
PB-VS	15,706	44	69.8	0.28	3.13	11
SB/PB-VS	220	34	54.0	15.45	167.07	0.71

time used is just about 1 min (0.017 h) on a desktop PC equipped with Intel E5420 (2500 MHz) processor if the molecular descriptors have been prepared (the time cost for the calculation of molecular descriptors of the M-MDDR compounds is about 5 h on the same computer). For PB-VS, the number of the predicted positives is 15,706, and that of the total hits is 44 with a yield of 69.8% (44 out of the 63 antagonists, see Table 3). The hit rate and enrichment factor are 0.28% and 3.13, respectively. The time cost for the screening of M-MDDR by PB-VS is about 11 h.

Then the multistep VS approach, in which the faster SB-VS is used firstly, followed by the slower PB-VS, was adopted to screen the M-MDDR library. The number of predicted positive compounds is 220, and that of total hits is 34 with a yield of 54.0% (34 out of the 63 antagonists, see Table 3). The hit rate and enrichment factor are 15.45% and 167.07, respectively, which are significantly higher than the corresponding values of individual VS methods. The total time used is about 0.71 h, which is significantly shortened compared with the sole use of PB-VS. All of these results show that the combined ligand-based VS approach outperforms the individual SB-VS and PB-VS (The established SVM model and pharmacophore hypothesis Hypo1 are freely available online at http://sklb.scu.edu.cn/lab/yangsy/download/mGluR1.rar).

Virtual screening by using the multistep VS approach for retrieving novel mGluR1 antagonists. The optimal VS method, namely the multistep VS approach, was used to screen several large chemical libraries including PubChem (18,831,686 compounds), Specs (202,408 compounds), and Enamine (980,000 compounds). Compounds (69, 256) passed through the first filter SB-VS. These compounds were next undergone the second filtering process by PB-VS, which prioritized them based on their fitness values. The top 200 compounds were taken as our final hits. After that, we performed a similarity analysis through calculating the Tanimoto coefficients (Tc) by using ECFP_4 fingerprint[32] between the final hits and the known antagonists. The results show that 195 of the 200 hits (97.5%) have a Tc value larger than 0.2 (see Table S5 in Supplementary data); in general, a Tc value larger than 0.2 of compounds with the known active agents is thought as a prerequisite for potential active compounds.[33–35] This increases to some extent our confidence in the screening results. Finally, we selected a total of 20 compounds from the 200 hits based on a visual look as well as our experience to purchase from the market. These compounds have been shifted to in vitro and in vivo studies, which results will be reported in the near future.

In summary, this study established a multistep ligand-based virtual screening approach, namely SB/PB-VS, based on SVM and pharmacophore models for discovering potent mGluR1 antagonists. The SB/PB-VS was demonstrated to be able to significantly increase the hit rate and enrichment factor compared with the individual SB-VS and PB-VS methods. Another advantage of the multistep VS method is the high speed in screening large chemical libraries. In addition, numerous hit compounds obtained by using the multistep VS method in screening several large chemical databases were also offered here. Overall, this study provides a good reference for the discovery of lead compounds whose targets are unknown or their crystal structures are unavailable, typically for example, family C GPCRs.

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Supplementary data

Supplementary data (chemical structures and bioactivities of the training and validation sets for the SVM classification model of mGluR1 antagonists and non-antagonists (Table S1, S2), chemical structures and bioactivities of the independent test set (Table S3), an interpretation for each of the 40 selected descriptors (Table S4), the top 200 compounds obtained by using the multistep VS method (Table S5), methodology) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.087.

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