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Scaffold Hopping and Optimization towards Libraries of Glycogen Synthase Kinase-3 Inhibitors

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Abstract—Using a virtual screening strategy based on a methodology derived from the CATS molecular descriptor, a novel compound class with inhibitory activity against the GSK-3 enzyme was identified through scaffold hopping. These compounds were readily synthesized, either by solid-phase or solution-phase chemistry. Compounds with inhibitory activity below 1 μM were identified.

Glycogen Synthase Kinase-3 (GSK-3) is a protein-serine kinase implicated in the hormonal control of several regulatory proteins. It was first discovered by virtue of its ability to phosphorylate and inactivate glycogen synthase, the regulatory enzyme of glycogen synthesis in mammals.[1,2] Since then a number of other substrates have been identified, implicating the enzyme in the regulation of several physiological processes. A number of different chemical compounds have recently been found to inhibit the GSK-3 kinase, for example, indirubins,[3] paullones,[4] maleimide derivatives[5,6] and the marine sponge hymenialdisine.[7] These compounds belong to highly different structure classes and show varying degrees of kinase specificity.

Compounds that specifically inhibit GSK-3 activity may be useful in the treatment of diabetes. To find new inhibitors of the GSK-3 kinase the Novo Nordisk library of compounds was screened for GSK-3 activity using standard HTS technology. The results derived from these measurements were single point determinations representing the amount of GSK-3 activity left within each sample after treatment with the test compound. Forty-seven active compounds were identified as a source for a drug discovery project. However, only few of these hit compounds were suitable for further optimization. To find better hits, a scaffold hopping approach was attempted. Scaffold hopping can be defined as the identification of isofunctional molecular structures with significantly different molecular backbones.[8] In this study, we have compared 32 different virtual libraries against all 47 GSK-3 hits derived from HTS. Using the in-house capability of making 'cherry-picked' library designs within a virtual scope based on verified chemistry and immediately available reagents all virtual compounds were readily available from solid-phase parallel synthesis.

CATS2 Analysis and Compound Library Design

In traditional similarity analysis based on 2D fingerprints, it is often assumed that compounds above a Tanimoto similarity of 0.85 relative to a known active reference are likely to be active.[9] Using this technique,[10] we found no structural similarity between any of the 47 GSK-3 hits and all 137,847 enumerated molecules from the virtual scopes as shown in Figure 1. Thus, no virtual molecules could be selected for synthesis by this approach.

Recently, Chemically Advanced Template Search (CATS) has been developed as a technique for virtual screening.[8] This approach calculates similarity between molecules by comparing the topological pattern of pharmacophore features assigned to atom pairs. An analogous (CATS2) methodology has been implemented in our laboratory combining components from the SPL[10] and Perl[11] computer scripting languages. Differences from the original implementation are mainly related to the definition and assignment of the different pharmacophore atom features within a molecule.[12] These pharmacophore atom features are Positive,

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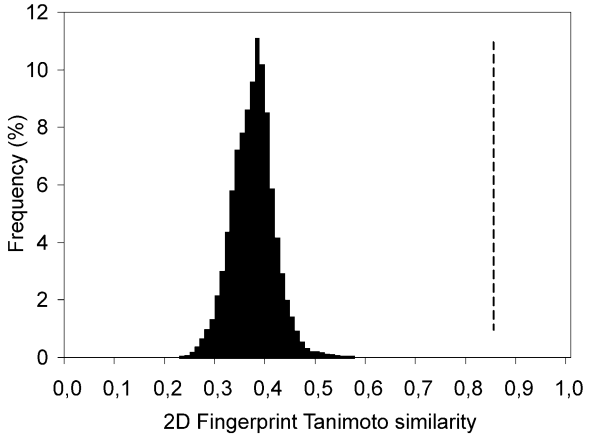


Figure 1. Lack of similarity between the 47 HTS hits and all potentially synthetically accessible compounds available from 32 virtual libraries.

Negative, Acceptor, Donor and Hydrophobic. Additionally, we have chosen to reduce the overall lipophilic contribution to the molecular mapping by excluding lipophilic–lipophilic atom pairs.

Computationally, all virtual libraries were compared simultaneously against each of the 47 HTS hits by using the CATS2 approach (Table 1). Based upon the hit rate found after virtual screening, the library number V14 was selected. Then, the 821 hits found by CATS2 were further reduced to 324 by conventional fragment-based 2D fingerprint diversity analysis in order to represent as many different molecular fragments as possible within the final library design.

A combinatorial library was synthesized according to Scheme 1 and tested in the GSK-3 assay as a single-point determination at 30 μM concentration. IC_{50} values were only determined for inhibitors showing less than 60% activity left. Compounds with activity above 100% may be activators of GSK-3, but this issue remains unexplored. Several compounds from this 'cherry-picked' library, derived from 14 R1, 8 X and 41 R2 fragments, displayed significant inhibition (Fig. 2) and the most active compound (**1**) is shown in Table 2.

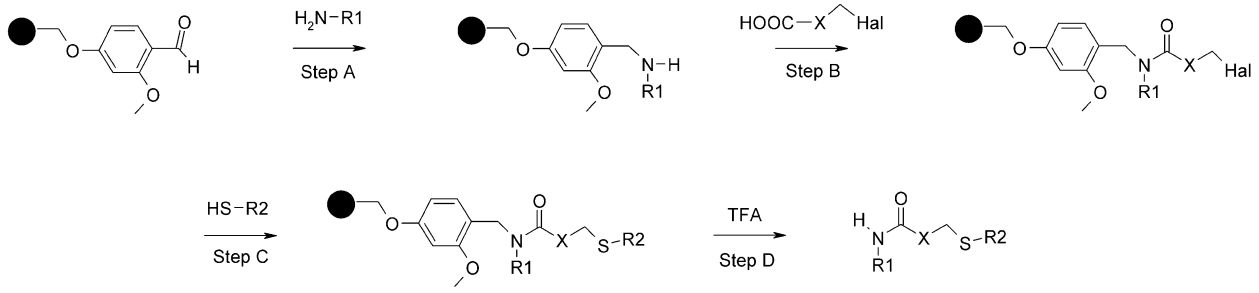
It is tempting to analyze the resulting molecule in terms of recognizable fragments from the parent HTS hit (Table 2) but, even though one can visually identify certain common molecular features, it must be stressed that CATS generates composite descriptors for the whole molecule and similarity is really measured in terms of dissimilarity. The two molecules shown are similar because they have less global dissimilarity and hence one cannot specify any structural part as being particularly similar to another. Nor can one infer a common binding mode.

Table 1. CATS2 similarity searching results within the virtual scope derived from 32 libraries

Library number	Compounds in virtual scope	Compounds examined by CATS2[a]	Hits found using CATS2[b]
V1	3432	3279	6
V2	7800	874	0
V3	400	400	0
V4	17,640	3989	0
V5	5775	2094	0
V6	28,729	3549	0
V7	9044	4021	0
V8	12,960	6008	0
V9	768	333	0
V10	1178	1003	0
V11	1892	1881	0
V12	2160	2047	0
V13	11,880	10,000	0
V14	9450	8622	821
V15	84,000	5118	0
V16	55,200	8573	0
V17	3283	1376	0
V18	861,224	10,000	0
V19	10,952	7142	0
V20	11,070	10,000	0
V21	55,125	5593	0
V22	1963	1068	0
V23	26,312	4416	0
V24	18,000	3288	0
V25	2891	708	0
V26	5148	2118	69
V27	7128	6984	0
V28	113,680	10,000	109
V29	1681	1680	0
V30	2352	2341	0
V31	1,248,884	9269	0
V32	42,840	73	0

[a]Filtering criteria were $M_r < 500$ and a maximum of 10,000 randomly selected compounds.
[b]A Euclidian distance < 0.25 in descriptor space was used as cut-off.

A crude SAR obtained by evaluation of this library indicates that the R2 fragment (Scheme 1) as a 2-substituted 5-(1-pyridyl)-[1,3,4]-oxadiazol moiety is important for activity. Additionally, a one-carbon linker between the sulfur atom and a phenyl ring seems to be important. In order to further optimize the compounds derived from the library, a computational HQSAR[10]



Scheme 1. Synthetic route towards the libraries.[14]

model was established using standard set-up and validation. This model can predict the activities of virtual compounds within the scope of library V14.

With this information in hand, a new compound library including the compounds predicted as the most active was synthesized according to Scheme 1 and tested in the GSK-3 kinase assay. This 'cherry-picked' compound library was biased towards compounds containing the oxadiazol-pyridyl moiety and comprised of 96 compounds derived from 21 R1, 1 X and 15 R2 fragments. This library included an increased number of active compounds, but unfortunately no compound was found to be more potent or efficacious compared to the first compound library (Fig. 2). However, this library of compounds confirmed the importance of the oxadiazol-pyridyl moiety to obtain active compounds.

Further Synthesis and SAR

The new compound series was well suited for single array synthesis of compound libraries in solution phase.

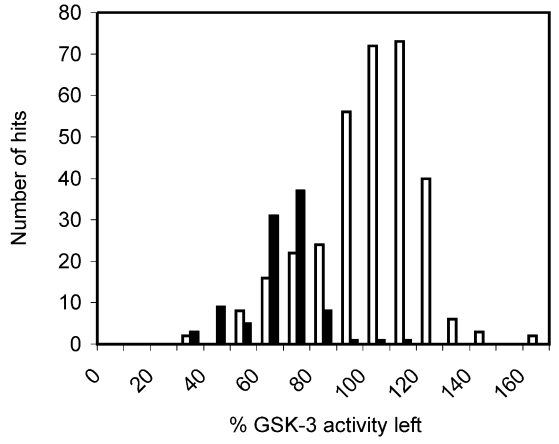
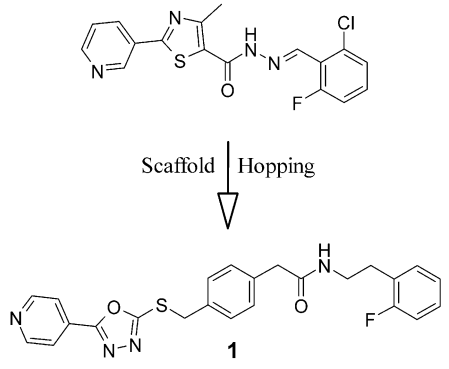


Figure 2. Hit rate and activities measured from the library containing 324 compounds (white) and the second optimized library containing 96 compounds (black). Low activity values represent active compounds.

Table 2. The most potent library compound (**1**) and the corresponding HTS hit



1

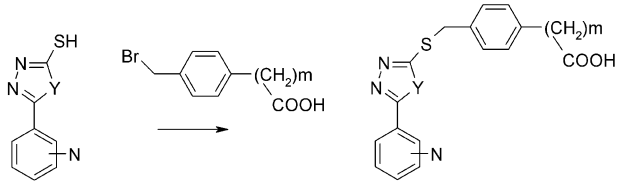
Compd	GSK-3 IC_{50} (μM)[13]
1	1.2

A general procedure for the synthesis was used.[15] More than 100 compounds were prepared by this method and tested in the GSK-3 kinase assay. The most important SAR information extracted from these data is given in Tables 3 and 4. Early on, it became clear that the R1-NHCO fragment within the solid-phase synthesized library was of limited importance for the activity. To reduce the number of flexible bonds and the molecular weight, for the compounds prepared in solution phase, the R1-NHCO fragment was replaced by smaller substituents directly attached to an aromatic ring.

As seen from the data in Table 3, a 4-pyridyl moiety is crucial for activity. A sulfur or oxygen in the five-membered heterocycle gives compounds with similar potency, whereas an H donor at the same position is detrimental for the activity. The size and bulkiness of the substituent on the aromatic ring seems to be of minor importance.

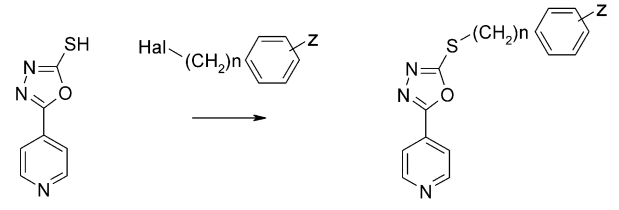
Additionally, the optimum substitution pattern for the aromatic ring was examined. As shown in Table 4, compounds with substituents in the 3-position consistently attained the best inhibitory activity.

Table 3.



Compd	Positional isomer	*Y*, m	GSK-3 IC_{50} (μM)[13]
2	4-Pyridyl	S,1	11
3	4-Pyridyl	O,1	8.0
4	4-Pyridyl	NH,1	>250
5	3-Pyridyl	S,1	>250
6	2-Pyridyl	S,1	>250
7	4-Pyridyl	O,0	29

Table 4.



Compd	*n*	*Z*	GSK-3 IC_{50} (μM)[13]
8	1	H	6.7
9	2	H	29
10	1	3-Cl	0.82
11	1	2-Cl	12
12	1	3-I	0.39
13	1	4-I	198
14	1	3-F	1.4
15	1	3-$COOCH_3$	1.1
16	1	3-COOH	235
17	1	W[a]	>250

[a]W, 3-(5-methyl-1,2,4-oxadiazolyl).

Conclusion

Using a virtual screening strategy based on the CATS2 molecular descriptor, a novel compound class with inhibitory activity on the GSK-3 enzyme was identified by scaffold hopping where the conventional 2D fingerprint-based similarity method fails. These compounds were readily available either by solid-phase or solution-phase chemistry. Compounds with activities below 1 μM were identified. Some of these compounds are currently being evaluated in secondary screening assays.

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10. Software is developed and available from Tripos Inc., St. Louis, MO, USA.
11. Available from www.perl.org
12. Atom features as defined by the Sybyl Line Notation (SLN) language are: Acceptor N[not=NH]|O[not=OH], Donor Het [is=HetH], Negative O[is=O(H)Hev=Het], Positive N[(is=N (Any)(Any)Any&not=N-Hev=O)], and LipophilicC[not = C∼ N,C∼O,C∼S = O,C∼P=O]|S[not=SH, S∼O].
13. Inhibition of GSK-3 by a test compound was evaluated using human GSK-3β and a glycogen synthase derived substrate with the following amino acid sequence: YRR-AAVPPSPSLSRHSSPHQS(PO_4)EDEEE-NH_2. In brief, GSK-3β was incubated with 32 μM substrate and varying concentrations of test compound in a buffer containing 0.1 mM ^{33}P-labeled ATP, 10 mM magnesium acetate, 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.1% dithiothreitol and 0.03% Triton-X100 for 60 min at room temperature. The reaction was performed using 96-well filter plates. The reaction was terminated by filtration followed by addition of 25 μL 2% phosphoric acid to each well. All wells were then washed three times in 0.5% phosphoric acid to remove unreacted ^{33}P-labeled ATP, dried and radioactivity was counted in a Packard topcounter. Dose–response profiles were generated, and the IC_{50} value for inhibition of GSK-3 by the test compound was calculated.
14. Step A: To (4-formyl-3-methoxyphenoxy)ethyl-polystyrene (60 mg or less, 0.55 mmol/g) are added in the order given a solution of the primary amine (0.6 mmol) in a mixture of NMP (0.6 mL) and water (50 μL), $NaCNBH_3$ (0.6 mL of a 1 mol L^{-1} solution in THF), and acetic acid (0.12 mL). The mixture is shaken at room temperature for 5–10 h. Wash with methanol (1×1.5 mL) and NMP (2×1.5 mL). Step B: To the resin-bound amine (40 mg, 0.04 mmol) a solution of a halo acid (0.8 mmol) in DCP (0.7 mL) + NMP (0.7 mL) is added, followed by the addition of a mixture of DIC (0.062 mL, 0.4 mmol) and toluene (0.1 mL). The resulting mixture is shaken at room temperature for 5 h. Step C: After washing (2×1.5 mL NMP) a solution of the thiol (0.8 mmol) in NMP (1.4 mL) is added, followed by the addition of DIPEA (0.15 mL). The mixture is shaken for at least 12 h. Step D: Cleavage in neat TFA for 2–20 h.
15. To a solution of the thiol compound[16] (1 equiv) in dimethylformamide the appropriate benzylhalide (1 equiv) and powdered potassium carbonate (3 equiv) were added. The reaction was monitored by the disappearance of the yellow colour or by TLC. The reaction time was dependent upon the reactivity of the benzyl halide used and was generally in the range from 1 min to 1 h. Aqueous workup and neutralization of the reaction mixture with 1 N hydrochloric acid and isolation of the compounds by filtration gave products in excellent yield and purity. NMR and LC–MS data for the compounds were in accordance with the structures given.
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