Bioorganic & Medicinal Chemistry Letters 22 (2012) 591–596

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and combinatorial synthesis of a novel kinase-focused library using click chemistry-based fragment assembly

Takayuki Irie [a,*], Ikuo Fujii [b], Masaaki Sawa [a]

[a] Drug Discovery Research, Carna Biosciences, Inc., 3rd Floor, BMA, 1-5-5 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan
[b] Department of Biological Science, Graduate School of Science, Osaka Prefecture University, 1-1, Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

ARTICLE INFO

Article history:
Received 12 August 2011
Revised 2 October 2011
Accepted 22 October 2011
Available online 30 October 2011

Keywords:
Kinase inhibitors
Click chemistry
Triazoles
Kinase-privileged fragment
Fragment evolution

ABSTRACT

Fragment-based lead discovery is a new approach for lead generation that has emerged in the past decade. Because the initial fragments identified in the fragment screening typically show weak binding affinity, an intensive medicinal chemistry effort would be required to grow initial fragments into a potential lead compound. Here we demonstrate a kinase focused evolved fragment (KFEF) library, constructed by click chemistry-based fragment assembly, that is a valuable source of kinase inhibitors. This combinatorial assembly of two fragments, kinase-privileged alkyne fragments and diversified azide fragments, by two cycloaddition reactions shows a unique potential for the one-step synthesis of structurally diverse evolved fragments. The screening of this triazole-based KFEF library allowed the rapid identification of potent lead candidates for FLT3 and GSK3β kinase.

Protein kinases play key roles in the signaling pathways that regulate crucial cellular processes such as proliferation, differentiation and survival.[1] Deregulation of protein kinases has been implicated in a variety of diseases, and therefore the inhibition of protein kinases has emerged as an important target for drug discovery.[2]

Recently, fragment-based lead discovery has been used to find very small drug fragments as medicinal chemistry starting points,[3,4] and successfully delivered novel lead compounds for some kinase targets.[5] However, initial fragments identified in the fragment screening typically show weak binding affinity. Those fragments should be converted into hit or lead compounds (evolved fragments) with sufficient potency keeping smaller molecular weight and lower lipophilicity compared to traditional lead compounds,[6] and these evolved molecules would make the subsequent optimization step easier. In order to evolve the initial fragments into lead compounds efficiently, integration of other techniques such as X-ray crystallography, or nuclear magnetic resonance spectroscopy would be necessary to elucidate the binding modes of multiple fragments. Even having the structural data, an intensive medicinal chemistry effort would be still required to grow initial fragments or assemble multiple fragments into a potential lead compounds.

In this Letter, we describe the design and synthesis of a novel kinase focused evolved fragment (KFEF) library using the 'click chemistry' methodology to readily identify an evolved fragment compound as a lead candidate. Through a screening campaign of the constructed KFEF library against a panel of kinases, hit compounds having excellent inhibitory potency against FLT3 and GSK3β were identified. In addition, the kinase selectivity of hit compounds was discussed, and the possible binding mode in the active site of the enzyme was analyzed by computer modeling.

Protein kinases share high structural homologies within their catalytic domains, and most kinase inhibitors bind to the highly conserved ATP binding cleft via hydrogen bonds with the 'hinge' residues, which are considered as the most important binding interactions for the recognition of kinases. To incorporate the kinase hinge binding structural features into the KFEF library as kinase-privileged fragments, we extracted chemical structures that bind to the kinase hinge region from kinase inhibitors reported in the literature[7] (Fig. 1). To construct the KFEF library, we chose the alkyne-azide cycloaddition reaction known as 'click reaction'.[8,9] The triazole ring formed in this reaction produces planar structures,[10] which would fit to the narrow ATP binding site of kinases. Namely, the KFEF library is constructed from two fragment parts using click reactions: the kinase-privileged alkyne fragments and diversified azide fragments which could increase chemical diversity and allow for modification of drug-like properties, such as inhibitory potency, target selectivity, and physicochemical properties.

With a set of alkyne and azide fragments, we applied two cycloaddition reaction conditions: a typical Cu-catalyzed click reaction to obtain 1,4-disubstituted 1,2,3-triazoles, and a Ru-catalyzed

* Corresponding author. Tel.: +81 78 302 7040; fax: +81 78 302 7086.
E-mail address: takayuki.irie@carnabio.com (T. Irie).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.10.076

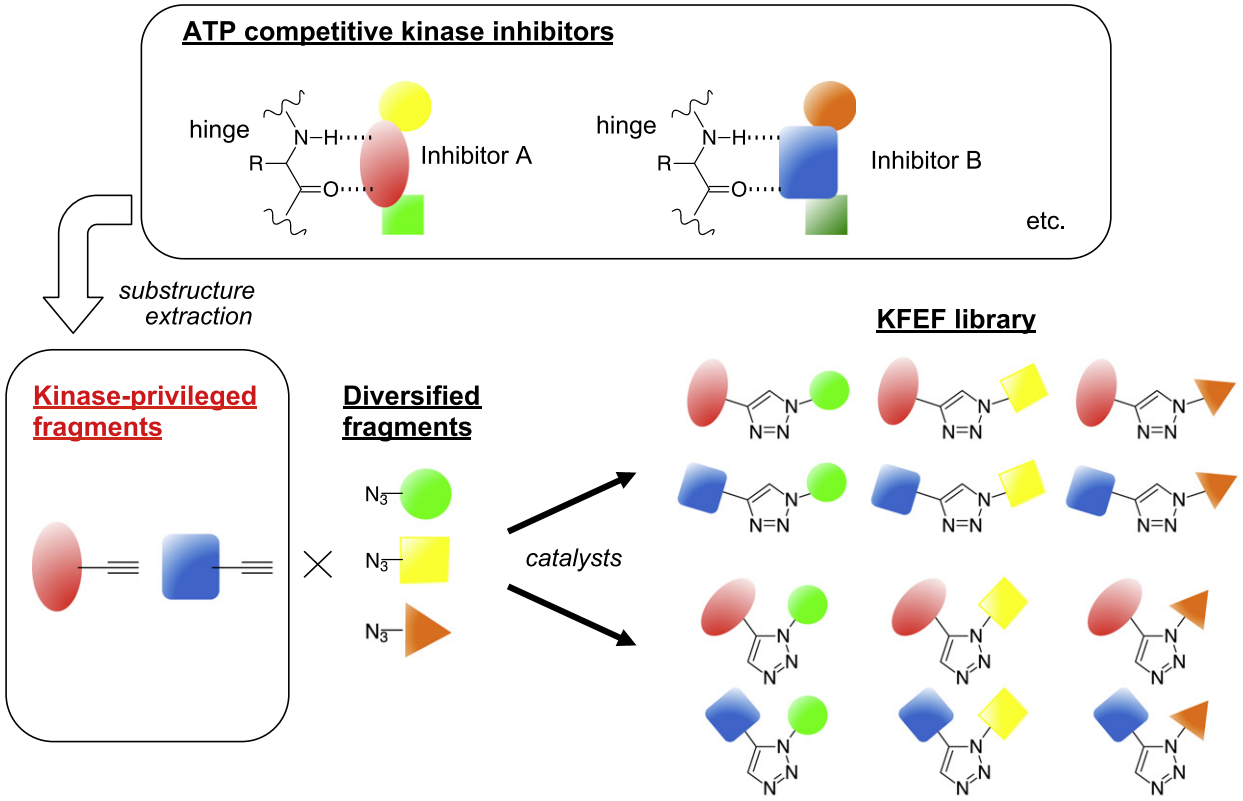
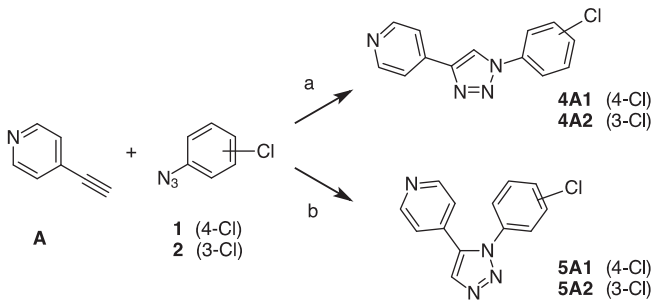


Figure 1. Design concept of KFEF library.

cycloaddition condition[11] to construct a 1,5-disubstituted 1,2,3-triazole library.

Before constructing the KFEF library, we performed a preliminary study to obtain an initial data set to confirm synthetic feasibility and the potential of a triazole structure to act as a kinase inhibitor, by screening against a panel of kinases. Based on the report by Graneto et al. that 1,5-disubstituted 5-membered ring analogs are potent MAP kinase inhibitors,[12] 4-ethynylpyridine **A**, possessing a nitrogen atom as a potential hydrogen bond acceptor, was employed as a privileged fragment.

4-Chlorophenyl azide **1** and 3-chlorophenyl azide **2** were chosen as diversified fragments which can be synthesized from corresponding anilines employing typical diazotization condition (Scheme 1). With these fragments, two 1,4-disubstituted triazoles (**4A1** and **4A2**) were synthesized using a standard Cu catalyzed click reaction condition (cat. $CuSO_4$, sodium ascorbate, *t*-BuOH/H_2O; RT, 1 day).[13] Two 1,5-isomers (**5A1** and **5A2**) were also prepared using the Ru catalyzed cycloaddition conditions reported by Fokin group (cat. $(Cp^*RuCl)_4$, DMF; microwave heating, 110 °C, 20 min).[14] Both 1,4-isomers and 1,5-isomers were successfully obtained in a regioselective manner[15] and the structures were confirmed by ^1H NMR (400 MHz, DMSO-d_6)[16]: the chemical shifts of H-5 in 1,4-disubstituted triazoles were appeared at lower field (~9.6 ppm) than those of H-4 in 1,5-disubstituted triazoles (~8.3 ppm).



Scheme 1. Regio-selective synthesis of 1,4- and 1,5-disubstituted 1,2,3-triazoles. Regents and conditions: (a) 1 mol % $CuSO_4$, sodium L-ascorbate (0.1 equiv), *t*-BuOH/H_2O (1:1), rt, 1 day; (b) 2.5 mol % $[Cp^*RuCl]_4$, DMF, microwave, 110 °C, 20 min.

To test their inhibitory activity for kinases, the four triazoles (**4A1**, **4A2**, **5A1**, and **5A2**) were screened against a panel of 46 kinases at 10 μM concentrations, and the percent inhibition against each kinase is expressed colorimetrically (Fig. 2). It is noteworthy that 1,4-disubstituted triazoles demonstrated potent inhibition primary against a subset of serine-threonine kinases (STKs) (DYRK1B, GSK3β, etc.) but were inactive against a majority of tyrosine kinases (TKs), while 1,5-disubstituted triazoles preferably bound to FLT3 (TK) with an excellent selectivity. Different patterns were observed between 1,4- and 1,5-regio-isomers, suggesting the KFEF library could span a wide range of the kinome tree with a small subset of fragments by using two click conditions.

As the initial concept of the KFEF library was validated, we next designed a small KFEF library composed of 5 alkyne and 8 azide fragments (a total of 80 compounds, Fig. 3). The library was prepared using the same synthetic procedure described above. Most of the reactions proceeded very well except azide **5** and **7** that might have poor reactivity, and the reaction did not produce **4D5**, **5C5**, **5B7**, and **5E7**. Thus, a total of 76 compounds successfully obtained (success rate = 95%) were subjected to screening to identify promising lead compounds against kinase targets.

FLT3 is a kinase responsible for survival and proliferation of leukemic blast cells. In acute myeloid leukemia (AML), constitutively activated mutations of FLT3 such as internal tandem duplications

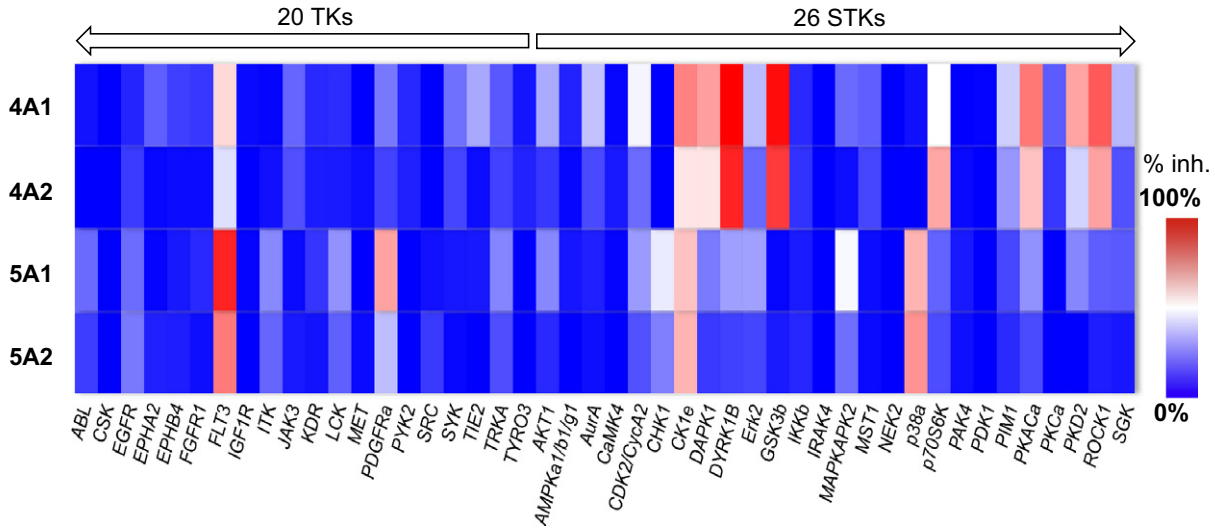


Figure 2. Protein kinase profiling of triazoles **4A1**, **4A2**, **5A1**, and **5A2**. The compounds were tested against a panel of 46 kinases. The color bar represents the percent inhibition at 10 μM concentrations.

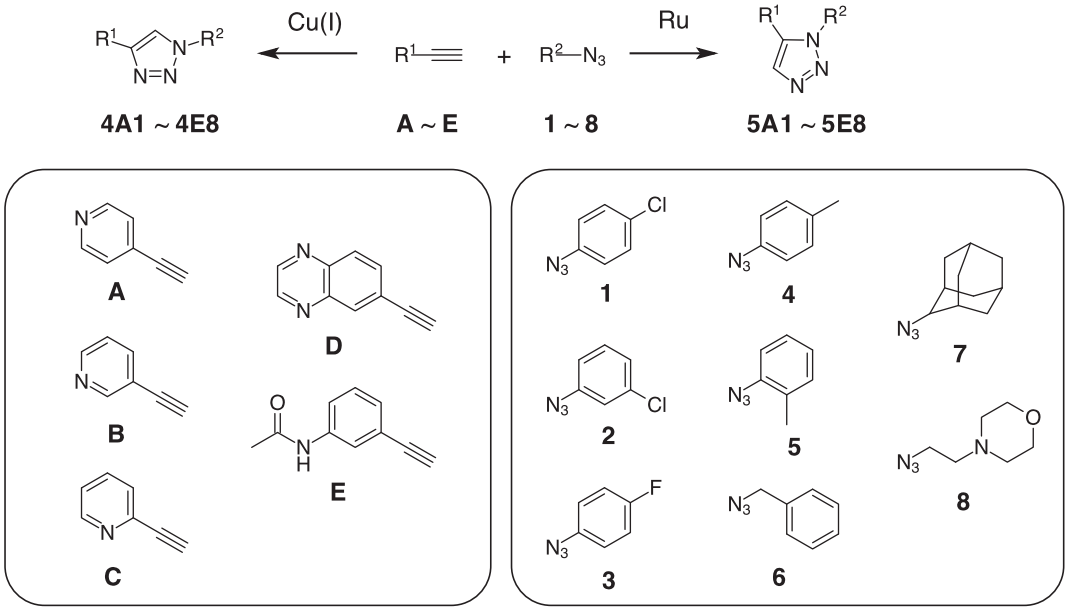


Figure 3. Click construction of 1,2,3-triazole KFEF library.

are found in up to 30% of the patients, and these FLT3 mutations are strongly associated with poor prognosis in AML.[17] On the other hand, GSK3β is one of the major kinases responsible for abnormal hyperphosphorylation of tau protein. Some studies suggested that this event would promote neurofibrillary tangle formation, which is involved in the pathogenesis of Alzheimer's disease (AD).[18] Accordingly, inhibitors of FLT3 or GSK3β have received much attention as potential drugs for AML or AD, respectively. Therefore FLT3 and GSK3β were selected as target kinases for further study based on the preliminary panel screening results.

A screening of the KFEF library was conducted against both FLT3 and GSK3β with the results shown in Figure 4. Apparently, there are clear differences in the hit patterns of the two kinases. For FLT3, 1,4-isomers incorporating the privileged fragments (**A**–**E**) generally showed weak or moderate inhibitory potencies similar to the initial compounds, **4A1** and **4A2** (Fig. 4, upper left). In contrast, 1,5-isomers exhibited a clear preference for the privileged fragment **A**, suggesting the 4-pyridyl group could be an excellent hinge binder for FLT3 (Fig. 4, upper right). On the other hand, some 1,4-isomers (**4A1**, **4A2** and **4C5**) showed strong inhibition against GSK3β while most of the remaining compounds showed no inhibition (Fig. 4, lower left). Interestingly, a small change in the nature of the para aromatic substituent in the R^2 group, 'Cl' (**4A1**) to 'F' (**4A4**) or 'Me' (**4A3**), dramatically diminished inhibitory potency, indicating a narrow window for activity as observed in some other kinase inhibitors.[19] Contrary to the preliminary screening results, some hit compounds (**5A5**, **5A7** and **5D7**) for GSK3β were identified within the 1,5-isomer library (Fig. 4, lower right).

The compounds showing >40% inhibition against each kinase in the screening were subjected to IC_{50} determination with the results shown in Tables 1 and 2. It can be seen from the results (Table 1) that the 1,5-isomers exhibited better potency against FLT3 compared with 1,4-isomers overall, as expected from the screening result at 10 μM concentration. However, 1,4-isomer **4C4** having a

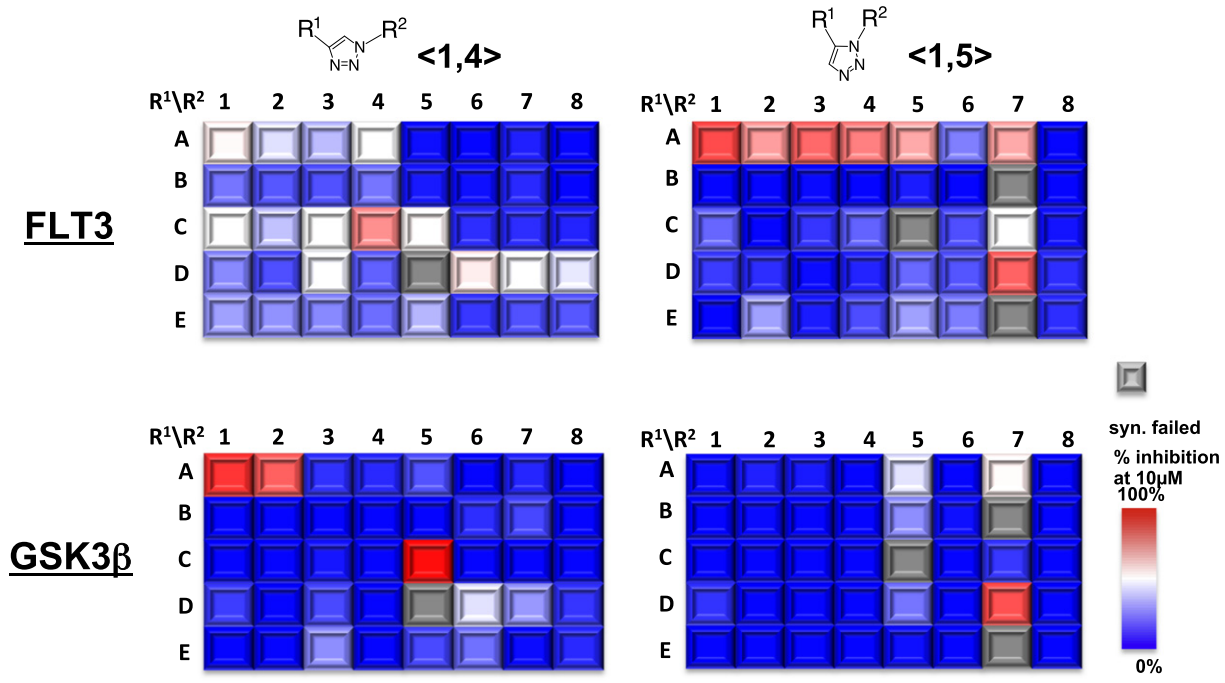


Figure 4. Inhibitory activity of KFEF library (left: 1,4-isomers; right: 1,5-isomers) against FLT3 (up) and GSK3β (down) at 10 μM concentration. To each cell was allocated a compound composed of an alkyne (A∼E) and an azide (1∼8). The percent inhibition is depicted colorimetrically according to the scale shown, while the synthetically unavailable in gray.

Table 1
IC_{50} values of hit compounds against FLT3

ID	R^1	R^2	IC_{50} (μM)	BEI[a]
1,4-Disubstituted triazoles				
4A1	4-Pyridyl	4-Chlorophenyl	5.8	20.4
4A2	4-Pyridyl	3-Chlorophenyl	10.5	19.4
4A4	4-Pyridyl	4-Methylphenyl	6.6	21.9
4C1	2-Pyridyl	4-Chlorophenyl	6.3	20.3
4C3	2-Pyridyl	4-Fluorophenyl	6.7	21.5
4C4	2-Pyridyl	4-Methylphenyl	1.1	25.2
4C5	2-Pyridyl	2-Methylphenyl	9.3	21.3
4D3	6-Quinazolyl	4-Fluorophenyl	12.4	16.8
4D6	6-Quinazolyl	Benzyl	6.3	18.1
4D7	6-Quinazolyl	2-Adamantyl	9.7	15.1
4D8	6-Quinazolyl	2-Morpholinoethyl	10.9	16.0
1,5-Disubstituted triazoles				
5A1	4-Pyridyl	4-Chlorophenyl	1.7	22.5
5A2	4-Pyridyl	3-Chlorophenyl	4.4	20.9
5A3	4-Pyridyl	4-Fluorophenyl	1.4	24.4
5A4	4-Pyridyl	4-Methylphenyl	2.3	23.9
5A5	4-Pyridyl	2-Methylphenyl	6.2	22.0
5A7	4-Pyridyl	2-Adamantyl	4.8	19.0
5C7	2-Pyridyl	2-Adamantyl	10.2	17.8
5D7	6-Quinazolyl	2-Adamantyl	3.1	16.6
Staurosporin			0.00018	20.9

[a] BEI (binding efficiency index) = −logIC50/MW (KDa).

Table 2
IC50 values of hit compounds against GSK3β

ID	R^1	R^2	IC50 (μM)	BEI[a]
1,4-Disubstituted triazoles				
4A1	4-Pyridyl	4-Chlorophenyl	1.1	23.2
4A2	4-Pyridyl	3-Chlorophenyl	1.8	22.4
4C4	2-Pyridyl	4-Methylphenyl	>30	—
4C5	2-Pyridyl	2-Methylphenyl	0.49	26.7
4D6	6-Quinazolyl	Benzyl	9.5	17.5
1,5-Disubstituted triazoles				
5A5	4-Pyridyl	2-methylphenyl	6.3	22.0
5A7	4-Pyridyl	2-adamantyl	2.6	19.9
5D7	6-Quinazolyl	2-adamantyl	2.8	16.8
Staurosporin			0.011	17.1

[a] BEI (binding efficiency index) = −logIC50/MW (KDa).

4-methylphenyl group at the R^2 position showed the best potency for FLT3 inhibition (IC_{50} = 1.1 μM) contrary to the expectation. In addition, the compound **4C4** showed no activity against GSK3β (IC_{50}: >30 μM, Table 2). Surprisingly, a shifting of the methyl groups from the 4-position to the 2-position resulted in a dramatic change in the inhibitory profile, namely the compound **4C5** exhibited potent inhibitory activity against GSK3β with an IC_{50} value of 0.49 μM but showed weak inhibition for FLT3 (IC_{50} = 9.3 μM) (Tables 1 and 2). Interestingly, the introduction of a bulky adamantyl group into the R^2 position of 1,5-isomers (**5A7** and **5D7**) resulted in an increase of the inhibitory activity for GSK3β maintaining FLT3 inhibition, implying an introduction of a bulky fragment might result in a non-selective kinase inhibitor library.

In order to evaluate the potential of these compounds as lead candidates, the binding efficiency indices (BEI) were calculated from their IC_{50} values and molecular weights (Tables 1 and 2), because the concept of BEI has been used as a parameter to prioritize successful fragments for lead optimization.[20–22] The most potent inhibitors (**4C4**: IC_{50} = 1.1 μM for FLT3 and **4C5**: IC_{50} = 0.49 μM for GSK3β) have excellent binding efficiencies (BEI = 25.2 and 26.7, respectively), which are superior to that of a potent non-specific kinase inhibitor, staurosporin (BEI = 20.9 for FLT3 and 17.1 for GSK3β), indicating that a screening of KFEF library would be a promising approach to identify a lead candidate rapidly.

To predict the binding modes of the hit compounds with GSK3β, **4A1** and **4C5** were computationally docked into GSK3β using LibDock (Fig. 5).[23] The docking study proposed that **4A1** would bind to GSK3β in a similar position to where ATP binds. The pyridine nitrogen from the privileged fragment, as expected, forms a

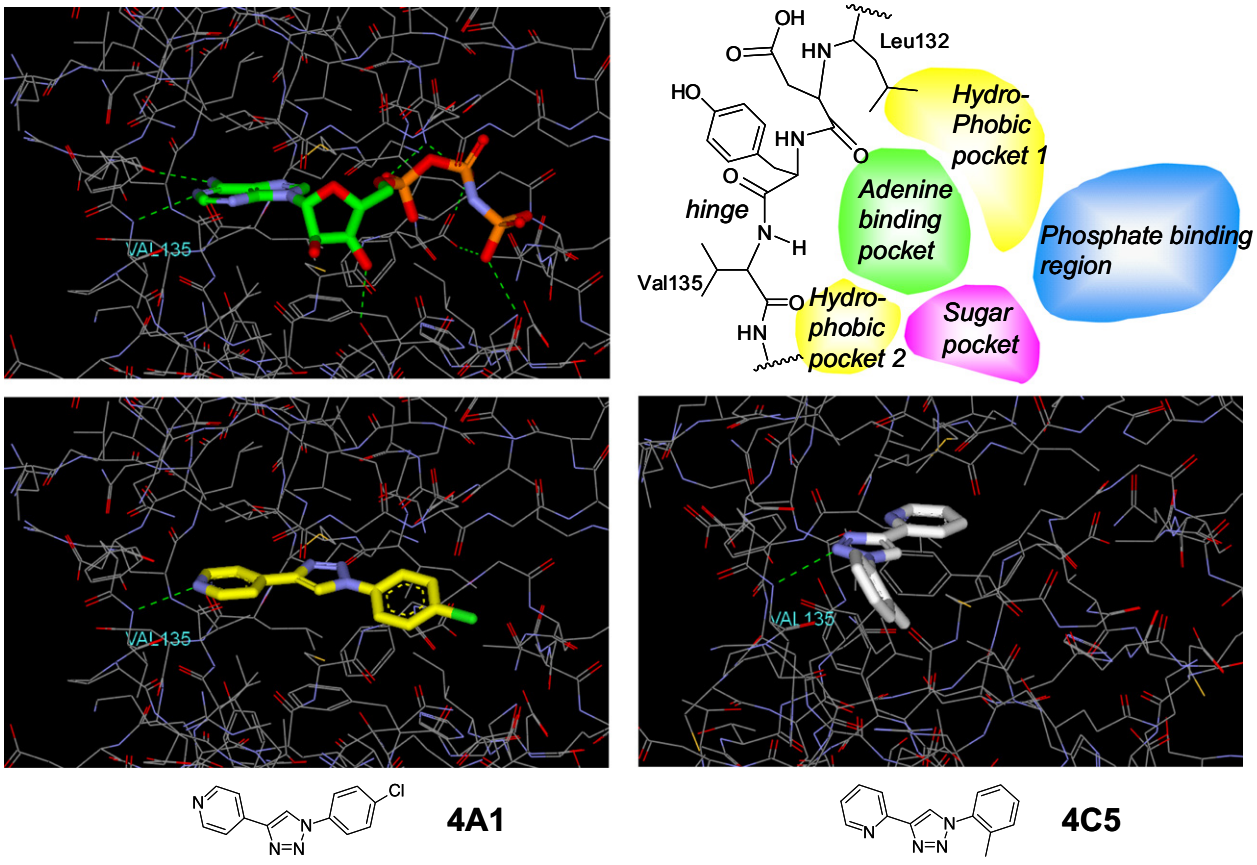


Figure 5. Docking study of hit compounds for GSK3β. (Upper) X-ray crystal structure of GSK3β with ATP–ANP (PDB code 1pyx) and a 2D representation of the ATP binding site. (Lower) **4A1** and **4C5** were computationally docked into GSK3β using LibDock. The crystal structure used in the docking study was retrieved from PDB (1pyx for **4A1** and 1q3d for **4C5**).

hydrogen bond with the hinge backbone amide. On the other hand, the binding mode predicted for **4C5** is very different from that of **4A1**. The triazole nitrogen can form a hydrogen bond with the hinge and therefore **4C5** could bind to the hydrophobic pocket 1 and 2.

In summary, 1,4- or 1,5-disubsituted 1,2,3-triazoles having kinase privileged fragments were designed and synthesized as a kinase focused evolved fragment (KFEF) library, and evaluated as potential kinase inhibitors. We have succeeded in hit generation for FLT3 (**4C4**, IC_{50} = 1.1 μM) and GSK3β (**4C4**, IC_{50} = 0.49 μM) by screening the KFEF library, and SAR obtained in this screening was beneficial for a further lead optimization step. This study reveals the potential of the KFEF library to be used in fragment-based drug design, and also provides a novel concept for design of kinase focused compound libraries.

Acknowledgments

We thank Mr. Fumio Nakajima for the docking study and the profiling team of Carna Biosciences, Inc. for the kinase profiling.

References and notes

1. Cohen, P. *Eur. J. Biochem./FEBS* **2001**, *268*, 5001.
2. Keri, G.; Orfi, L.; Eros, D.; Hegymegi-Barakonyi, B.; Szantai-Kis, C.; Horvath, Z.; Waczek, F.; Marosfalvi, J.; Szabadkai, I.; Pato, J.; Greff, Z.; Hafenbradl, D.; Daub, H.; Muller, G.; Klebl, B.; Ullrich, A. *Curr. Signal Transduct. Ther.* **2006**, *1*, 67.
3. Congreve, M.; Chessari, G.; Tisi, D.; Woodhead, A. J. *J. Med. Chem.* **2008**, *51*, 3661.
4. de Kloe, G. E.; Bailey, D.; Leurs, R.; de Esch, I. J. P. *Drug Discovery Today* **2009**, *14*, 630.
5. Erlanson, D. A. In *Kinase Inhibitor Drugs*; Li, R., Stafford, J. A., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2009; pp 461–483.
6. Rees, D. C.; Congreve, M.; Murray, C. W.; Carr, R. *Nat. Rev. Drug Disc.* **2004**, *3*, 660.
7. Ghose, A. K.; Herbertz, T.; Pippin, D. A.; Salvino, J. M.; Mallamo, J. P. *J. Med. Chem.* **2008**, *51*, 5149 (a review) and references therein.
8. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
9. Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
10. Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278.
11. Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005**, *127*, 15998.
12. Graneto, M. J.; Kurumbail, R. G.; Vazquez, M. L.; Shieh, H.-sheng.; Pawlitz, J. L.; Williams, J. M.; Stallings, W. C.; Geng, L.; Naraian, A. S.; Koszyk, F. J.; Stealey, M. A.; Xu, X. D.; Weier, R. M.; Hanson, G. J.; Mourey, R. J.; Compton, R. P.; Mnich, S. J.; Anderson, G. D.; Monahan, J. B.; Devraj, R. *J. Med. Chem.* **2007**, *50*, 5712.
13. Pagliai, F.; Pirali, T.; Del Grosso, E.; Di Brisco, R.; Tron, G. C.; Sorba, G.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 467.
14. Rasmussen, L. K.; Boren, B. C.; Fokin, V. V. *Org. Lett.* **2007**, *9*, 5337.
15. The reaction was monitored by HPLC-MS analysis (column: Unison US-C18, 4.6 mm i.d. × 50 mm; eluents: water/methanol with 10 mM HCOOH as gradient 90/10 to 10/90; flow rate: 2 mL/min; run time: 5 min), which was able to separate 1,4 and 1,5-disubstitued triazoles (e.g., RT = 2.7 and 2.9 min for **5A2** and **4A2**, respectively).
16. Satisfactory characteristics data were obtained for all new compounds in this manuscript. Characteristics are given for representative compounds: **4A1**, brown powder, 12% yield, ^{1}H NMR (400 MHz, DMSO-d_6) *d* 9.57 (s, 1H), 8.66–8.78 (m, 2H), 8.00 (d, *J* = 9.0 Hz, 2H), 7.83–7.93 (m, 2H), 7.75 (d, *J* = 8.8 Hz, 2H), ESI MS 356.8 $[M+H]^+$; **5A1**, brown amorphous, 16% yield, ^{1}H NMR (400 MHz, DMSO-d_6) *d* 8.58–8.67 (m, 2H), 8.35 (s, 1H), 7.62–7.71 (m, 2H), 7.46–7.57 (m, 2H), 7.25–7.35 (m, 2H), ESI MS 356.9 $[M+H]^+$.
17. Kiyoi, H.; Ohno, R.; Ueda, R.; Saito, H.; Naoe, T. *Oncogene* **2002**, *21*, 2555.
18. Hooper, C.; Killick, R.; Lovestone, S. *J. Neurochem.* **2008**, *104*, 1433.
19. Das, J.; Chen, P.; Norris, D.; Padmanabha, R.; Lin, J.; Moquin, R. V.; Shen, Z.; Cook, L. S.; Doweyko, A. M.; Pitt, S.; Pang, S.; Shen, D. R.; Fang, Q.; de Fex, H. F.; McIntyre, K. W.; Shuster, D. J.; Gillooly, K. M.; Behnia, K.; Schieven, G. L.; Wityak, J.; Barrish, J. C. *J. Med. Chem.* **2006**, *49*, 6819.
20. Tanaka, D.; Tsuda, Y.; Shiyama, T.; Nishimura, T.; Chiyo, N.; Tominaga, Y.; Sawada, N.; Mimoto, T.; Kusunose, N. *J. Med. Chem.* **2011**, *54*, 851.

21. Orita, M.; Ohno, K.; Warizaya, M.; Amano, Y.; Niimi, T. *Methods Enzymol.* **2011**, *493*, 383.
22. Abad-Zapatero, C.; Metz, J. T. *Drug Discovery Today* **2005**, *10*, 464.
23. The docking study was carried out by LibDock module of Discovery Studio 2.5 package (Accelrys, Inc., San Diego, CA). First, we set a docking sphere (radius 9 Å) that included the residues interacting the original ligands in the crystal structure. Next, ligand conformations generated automatically were docked within the sphere using LibDock by setting threshold energy as 20 kcal/mol. Finally, we extracted the docking poses which contain hydrogen bondings with the hinge reside (Val 135) and depicted them in Figure 5 using Discovery Studio Visualizer.