Strategies for the Design of Selective Protein Kinase Inhibitors

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Abstract: Most kinase inhibitors reported so far are designed to bind to a highly conserved ATP binding pocket in a competitive manner. In this case, inhibitors targeting the ATP pocket may crossreact with different kinases, as well as with other proteins that bind ATP, and this may cause undesirable side effects that would limit their clinical usefulness. In addition to the kinase selectivity issues, human ether-a-go-go-related gene (hERG) inhibition could be an obstacle to develop a kinase inhibitor as a safe drug. This paper will review the methods employed in the development of selective kinase inhibitors with several successful examples. These include medicinal chemistry efforts to conquer hERG inhibition problems as sometimes seen in kinase inhibitor programs.

INTRODUCTION

Anticancer chemotherapy was thought to be a difficult challenge until a recent huge success of kinase modulating therapy. Recent development of molecular biology uncovered the importance of kinase signaling networks that regulate cellular activities such as proliferation and survival [1]. Deregulation of these kinase activities was found to be well associated with a magnitude of diseases states, particularly cancer [2]. Therefore, it was anticipated that specifically targeting cancer cells by modulating aberrant kinase activities, as known for "Targeted therapy concept", would be efficacious in cancer with fewer side effects than traditional cytotoxic drugs [3]. The first example of targeted therapy by a small molecule is imatinib mesylate (Gleevec®), which selectively inhibits BCR-ABL kinase activity, aberrantly activated fusion protein kinase leading to uncontrolled proliferation of the leukemia cells [4]. Clinical trials of imatinib demonstrated remarkable therapeutic effects that 90% of patients achieved complete haematological remission when this small molecule is used to treat patients with chronic myeloid leukemia (CML), and imatinib is a now established first-line therapy for CML. The success of imatinib has sparked interest in small molecule kinase inhibitors for many different indications range from cancer to inflammatory disorders. According to our present knowledge, over 250 compounds with kinase inhibitory activity against almost 100 different kinase targets are currently in the various stages of preclinical and clinical development. This paper will review the methods employed in the development of selective kinase inhibitors with several successful examples.

SELECTIVITY OF KINASE INHIBITORS

Until recently, it was thought that inhibitors specific for a single kinase were impossible to achieve due to the fact that the human protein kinase family consists of over 500 enzymes with very similar active sites. However, the success of imatinib, which selectively inhibits Abl kinase, has encouraged the search for specific inhibitors of protein kinases (Fig. (**1**)).

A first step toward the design of a selective kinase inhibitor is to identify potential off-target kinases precisely among 518 kinds of protein kinase, which are encoded in the human genome referred to as the kinome [1] (Fig. (**2**)). This so-called "kinase profiling" is typically performed by measuring its activity against a panel of kinases *in vitro* [5]. The kinase enzymes in the panel are most often selected based on sequence homology, which are most likely to share inhibitor sensitivity [6]. However, unexpected crossreactivities with kinases positioned very distantly in the kinome tree are also often observed, and therefore, it is important to select representative kinases from each branch of the kinome tree [5]. The kinase profiling of a compound not only give a clue for the design of selective inhibitors, but it is useful for target hopping strategy, namely information about a given compound's activity against other kinase family members as the starting point for a new project.

GATEKEEPER AND SELECTIVITY

Most kinase inhibitors reported so far are designed to bind to a highly conserved ATP binding pocket in a competitive manner. As shown in Fig. (**3**), a substrate ATP binds to the ATP binding pocket by hydrogen bonding with the hinge region connecting the N- and C-terminal lobes of the kinase catalytic domain. Majority of kinase inhibitors reported so far bind to this hinge region in a similar manner and are designed to have additional interactions with hydrophobic regions I and II (Fig. (**3**)). The hydrophobic region I commonly referred to as the selectivity pocket, because the size of a single residue in the interior of this region, termed the gatekeeper, has been shown to be a critical determinant of inhibitor sensitivity [7, 8]. Based on our in-house human kinome database, approximately 90 kinases possess a small threonine gatekeeper, and 190 kinases have a large methionine residue. Interestingly, 50% of tyrosine kinases have the threonine as the gatekeeper, but only 10% of serine-threonine kinases have the threonine. For this reason, serine-threonine kinases are not much sensitive to tyrosine kinase inhibitors.

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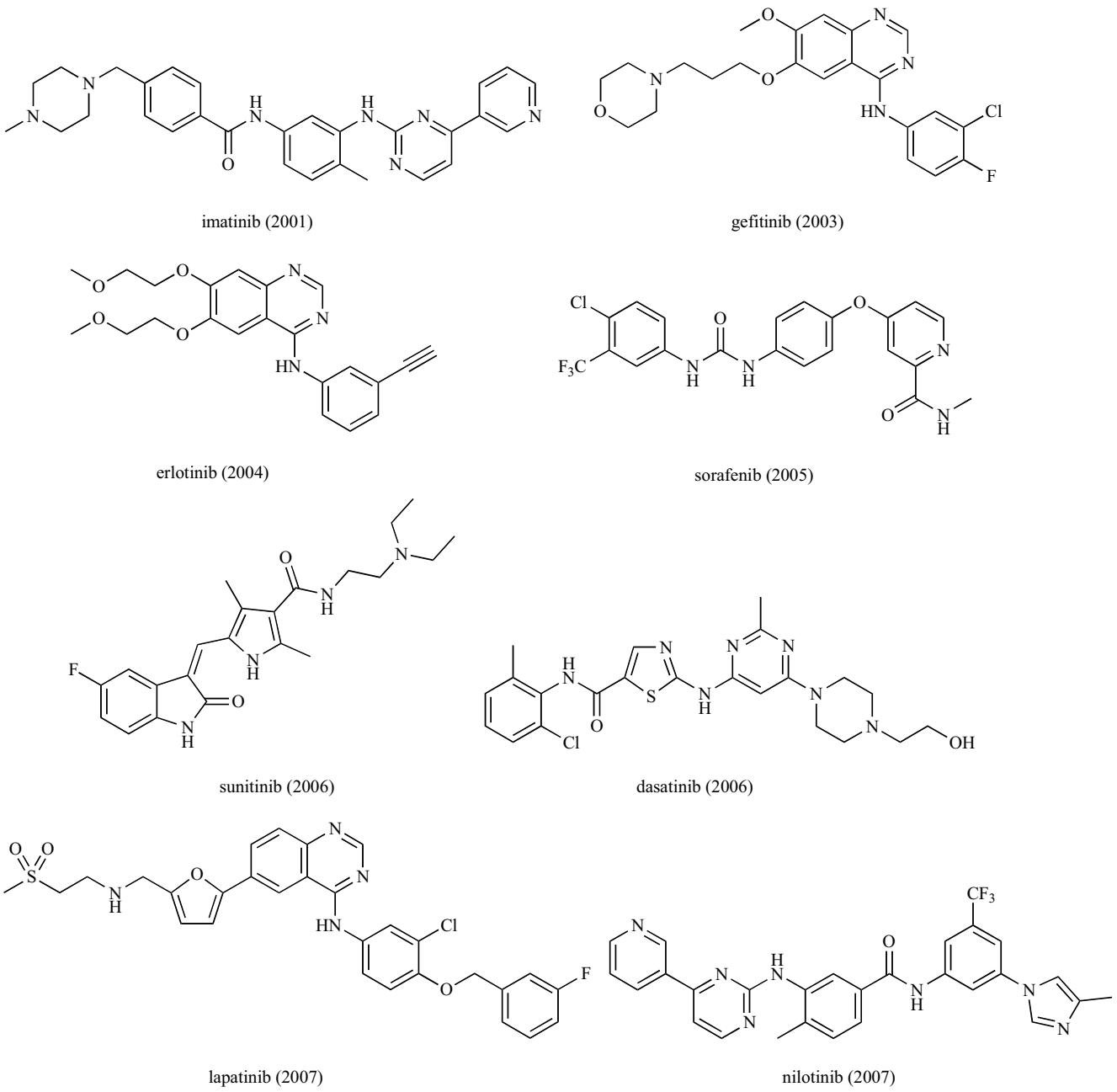


Fig. (1). Structures of kinase inhibitors approved by FDA.

Researchers from Novartis recently reported that the successful design of a selective fms-related tyrosine kinase 3 (FLT3) inhibitor through the interaction with the gatekeeper residues [9]. Docking studies of reported inhibitors with FLT3 suggested that a compound having an aryl ring able to make interactions with the gatekeepers Phe691 and Cys828 could have high inhibitory activity against FLT3 kinase (Fig. (**4**)). The designed inhibitor **1** proved to be a potent FLT3 kinase inhibitor (IC_{50} = 50 nM) as expected. It is noteworthy that compound **1** showed an excellent selectivity over a panel of kinases except few kinases possessing the same gatekeepers. The fact that compound **1** was identified using in silico design approaches suggests that targeting the gatekeeper residues may be a valuable strategy for the rational design of selective inhibitor.

Another example of a selective inhibitor targeting the gatekeeper was reported by researchers from Genomics Institute of the Novartis Research Foundation and The Scripps Research Institute recently [10]. A series of 4,6-diaminopyrimidine analogs were synthesized to identify a novel scaffold for kinase inhibitors because the 4,6-disubstitutions of pyrimidines are not examined well compared to 2,4-regioisomers. It was noted that only 4,6-isomer **2** was the potent inhibitor of Epidermal Growth Factor Receptor (EGFR) (IC_{50} = 21 nM) while other 2,4-isomers **3** and **4** showed no inhibition (Fig. (**5**)). Surprisingly 4,6-disubstituted pyrimidine **2**

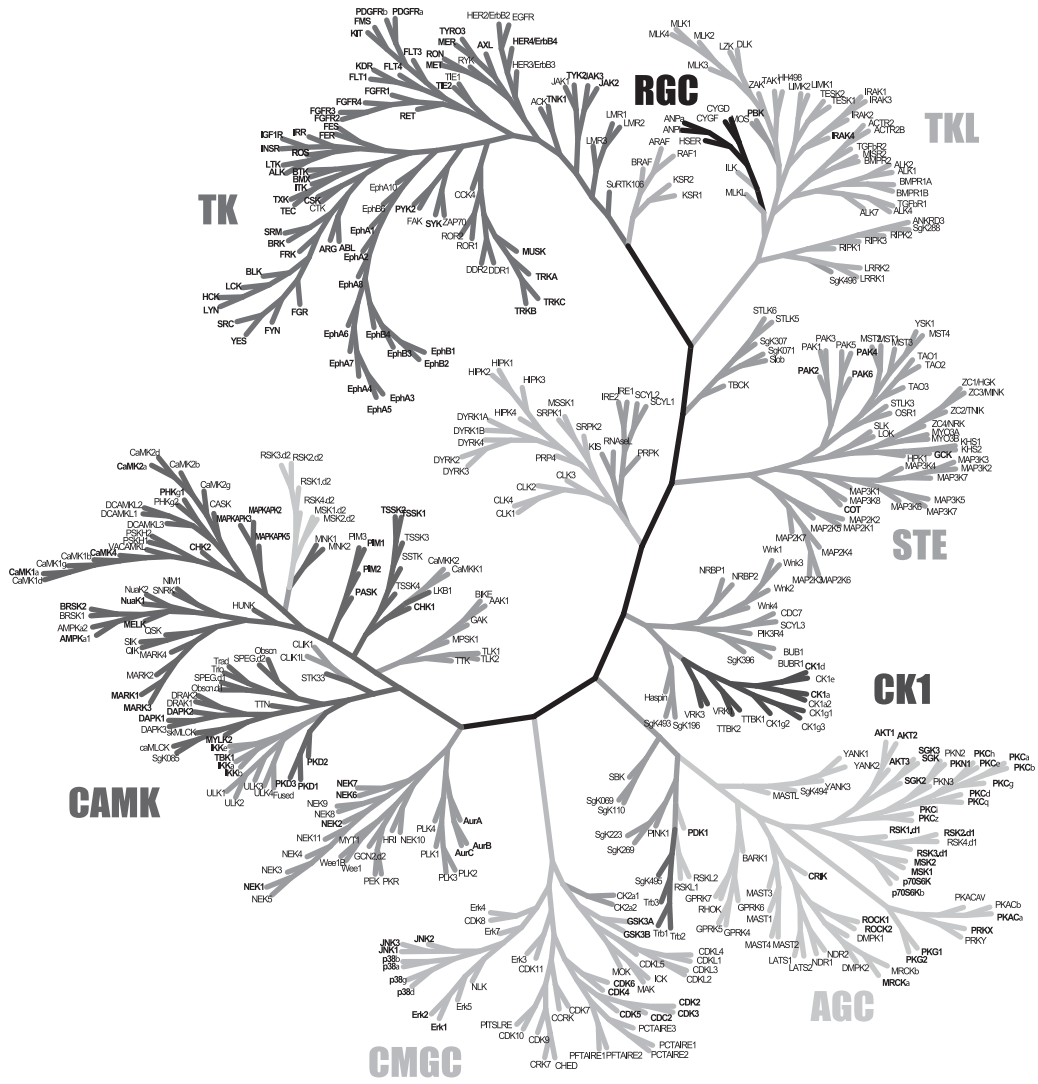


Fig. (2). The human kinome tree produced by Carna Biosciences, Inc. CAMK (calcium/calmodulin-dependent kinase group), TK (tyrosine kinase group), RGC (receptor guanylyl cyclase group), TKL (tyrosine kinase-like group), STE (sterile phenotype kinase group), CK1 (cell kinase 1/casein kinase 1 group), AGC (protein kinases A, G and C group), CMGC (cyclin-dependent-kinase (CDK), mitogen-activated-kinase (MAPK), glycogen-synthase-kinase (GSK) and CDK-like kinase group).

exhibited exclusive selectivity against EGFR over a panel of 55 kinases. Molecular modeling studies suggested that the hydrogen bond between N3 of pyrimidine core and the hydroxyl group of the threonine gatekeeper would be a key interaction for this high selectivity.

TARGETING INACTIVE CONFORMER

Activity of most kinases is regulated by changing structural conformations, active and inactive forms, and the phosphorylation of key residues in the activation loop (the DFG motif) can shift the balance between these states [11] (Fig. (**6**)). It is believed that structures of inactive conformations are much diverse than that of the active form [12]. Thus, the inactive forms of kinases are attractive targets to obtain highly selective inhibitors, so-called type II inhibitors. In fact, imatinib, which binds to the inactive form of Abl kinase, shows higher selectivity than inhibitors binding to the active Abl (type I inhibitors) [13]. Despite the benefit of targeting the inactive kinases, the majority of kinase drug discovery programs do not consider the conformation difference. Because all inactive kinase binders reported to date have been only discovered serendipitously by high-throughput screening (HTS) or during lead optimization, and later confirmed by co-crystal structure studies. Thus, producing selective inhibitors by targeting inactive conformations could be very challenging.

Okram and co-workers demonstrated that strategy for rationally designing type II inhibitors [14]. The concept for the rational design of type II inhibitor is to simply install the

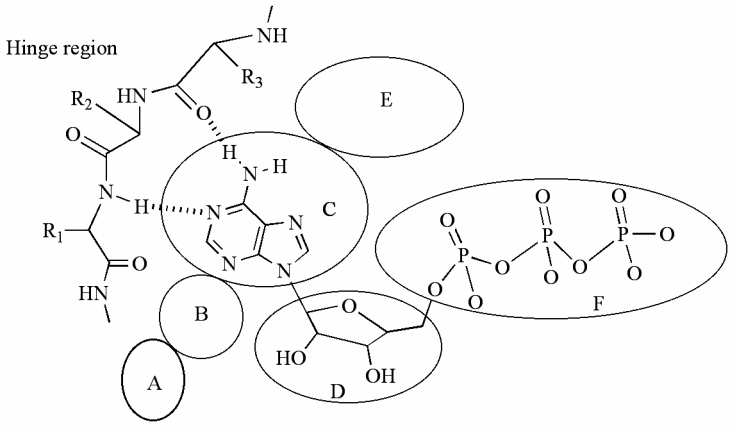


Fig. (3). Schematic representation of ATP binding pocket in protein kinases.

A: solvent accessible region, B: hydrophobic region II, C: adenine binding region, D : sugar pocket, E: hydrophobic region I, F: phosphate binding region.

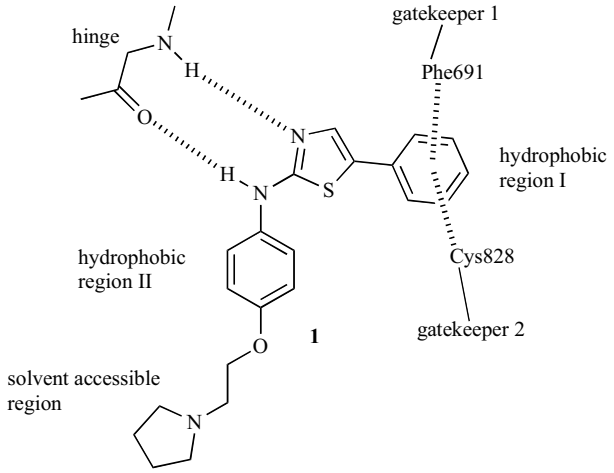
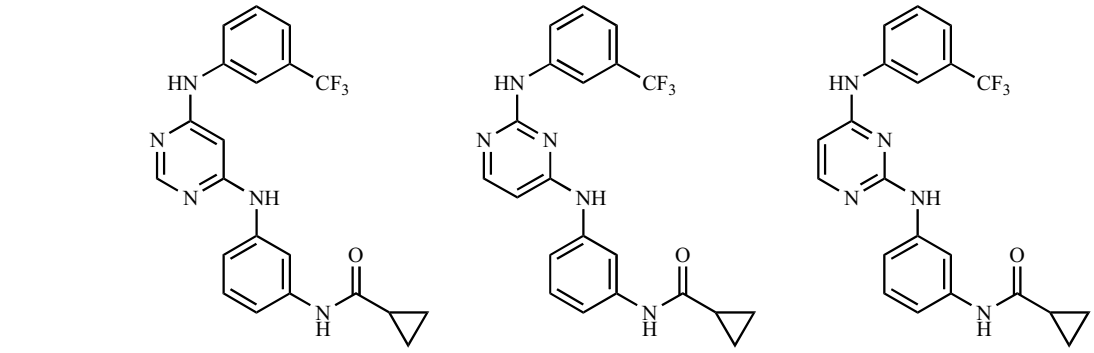


Fig. (4). Proposed binding interaction of compound **1** in FLT3. Hydrogen bondings between the aminothiazole part and hinge region were observed in the docking model. The phenyl ring would provide key interactions with two gatekeepers Phe691 and Cys828.



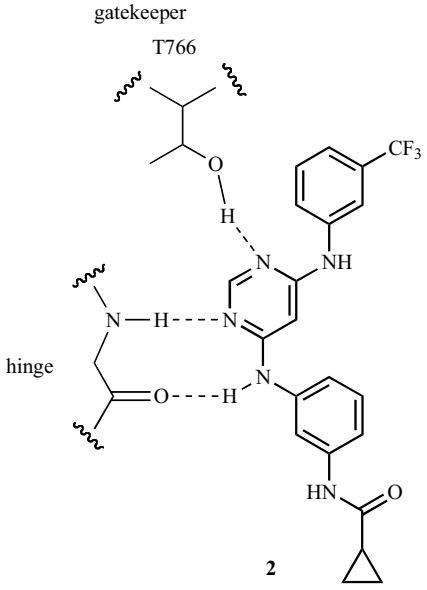


Fig. (5). Structures of pyrimidines and proposed binding interaction of compound **2** in EGFR. The model suggested the pyrimidine N3 could form a key hydrogen bonding interaction with the gatekeeper Thr766 in the ATP site of EGFR.

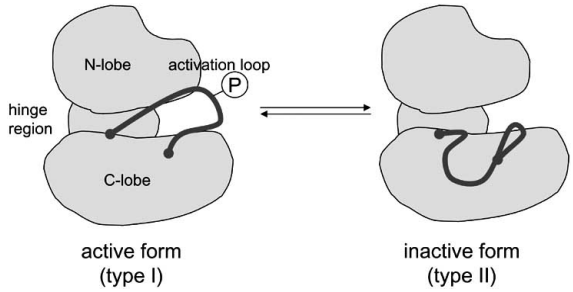
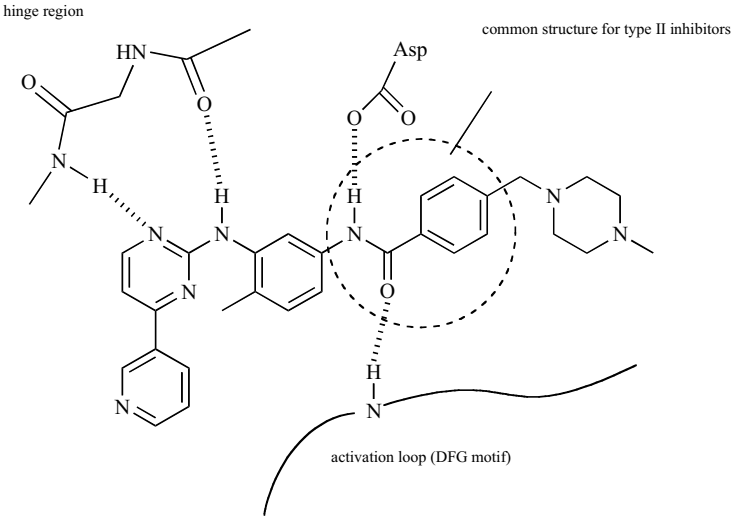
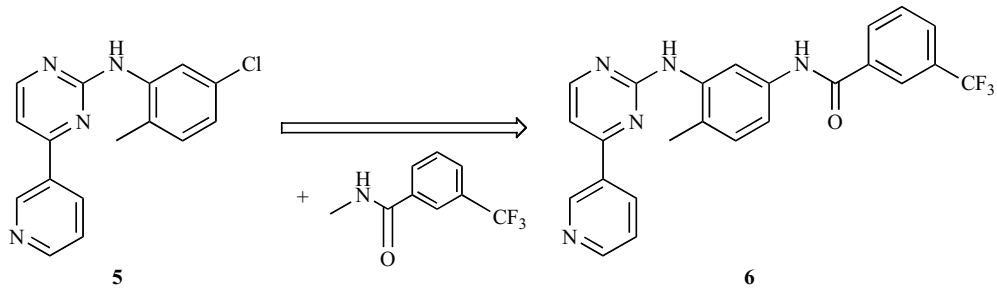


Fig. (6). Schematic representation of the equilibrium between active and inactive kinase conformations.

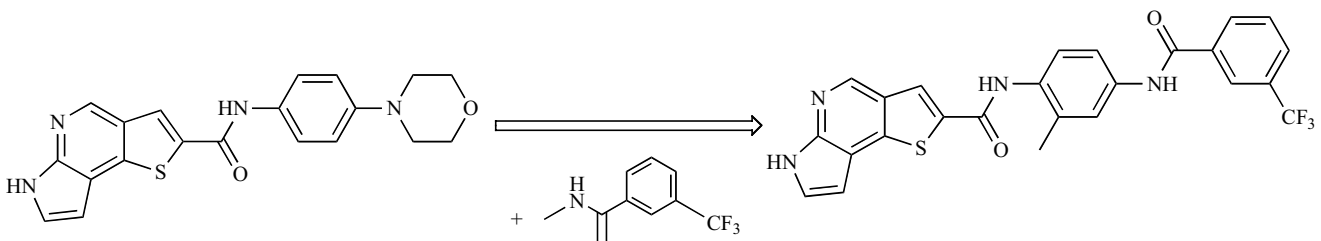


Type I ***Type II***



5 **6**

Ki (nM)	Abl (A)	Abl (I)	Abl (A)	Abl (I)
	>5000	>5000	152	5



7 **8**

Ki (nM)	Abl (A)	Abl (I)	Abl (A)	Abl (I)
	>5000	>5000	>5000	154

Abl (A); Active Abl
Abl (I); Inactive Abl

Fig. (7). Conversion of type I inhibitors into type II inhibitors. A 3-trifluoromethylbenzamide substituent was introduced into type I scaffolds (**5** and **7**) to generate type II inhibitors.

key structures "3-trifluoromethylbenzenamide" extracting from known type II inhibitors into type I scaffolds (Fig. (**7**)). Surprisingly this simple hybrid-design approach resulted in transformation of type I scaffolds into compounds that preferentially interact with inactive Abl kinases. The crystallographic study revealed that the hybrid compound binds to the inactive conformation as suggested by biochemical data. These results suggested that it is possible to produce type II inhibitors by medicinal efforts and this approach might accelerate the development of selective kinase inhibitors.

hERG INHIBITION

Cardiovascular toxicity is one of the most serious problems during a drug development. Drugs binding to hERG channels would cause QT interval prolongation, which would result in the withdrawn from the market [15]. Recently, there has been significant progress in the structural understanding of drugs that bind to hERG channels and the relationship between hERG binding potency and preclinical QT prolongation *in vivo* [16]. Ekin *et al*. generated a hERG pharmacophore model with the reported hERG inhibitors, which contained four hydrophobic features and one positive ionizable feature [17].

Majority of kinase inhibitors has an aminoalkyl functional group as a solubilizing group to compensate its hydrophobic scaffold property. Such structural features; a hydrophobic structural core with one basic nitrogen-containing pendant would be matched well with the Ekin's hERG pharmacophore model, and thus kinase inhibitors having a solubilizing group could be potent hERG inhibitors [18-23].

Researchers from Abbott Laboratories recently reported that the optimization efforts to decrease affinity for the hERG channel by modulating side chain basicity [23]. A series of 1,4-dihydroindeno[1,2-c]pyrazoles were identified as novel KDR kinase inhibitors, however the compounds were also good hERG channel inhibitors (Fig. (**8**)). They constructed a homology model of the homo-tetrameric pore domain of hERG to examine key interactions between compound **9** and hERG. The binding model suggested that 1,4-dihydroindeno[1,2-c]pyrazole core could make the π-stacking interaction with Phe656, and the two nitrogen atoms in the N-methylpiperazine-containg side chain could interact with Tyr652 and Ser624, respectively. Based on this model, the basicity of either nitrogen atom was modulated by introduction of neighboring carbonyl groups to reduce the interactions with Tyr652 or Ser624. As expected, both modifica-

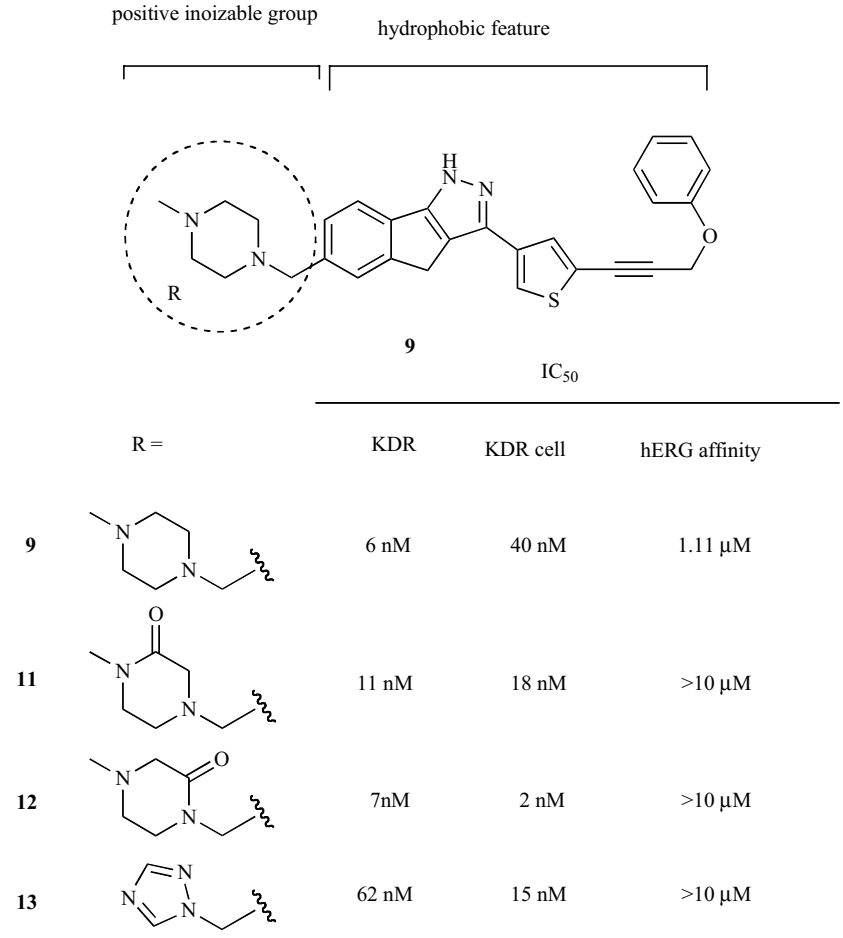


Fig. (8). KDR inhibitory activity and hERG affinity of 1,4-dihydroindeno[1,2-c]pyrazoles. Compounds were evaluated for the inhibition of *in vitro* kinase activity (KDR), intracellular KDR phosphorylation (KDR cell), and hERG channel affinity ([3H]dofetilide membrane binding assay).

tions led to a loss of hERG activity maintaining KDR activity. Replacements with aromatic heterocycles were also successfully reduced hERG activity with acceptable KDR activity, especially the 1,2,4-triazole analog demonstrated potent inhibition both in cell and *in vivo*. Unfortunately this 1,2,4-triazole analog showed poor pharmacokinetic profile.

CONCLUDING REMARKS

Human genome encodes over 500 protein kinases that are involved in regulating complex cellular functions. Perturbation of protein kinase activity is linked to a number of diseases, such as cancer, diabetes, inflammation, and cardiac diseases, and therefore, protein kinases have become one of the major therapeutic targets of the past 10 years [24]. In fact, kinases have become the second most exploited group of drug targets after G-protein-coupled receptors (GPCRs) at present. Various small molecule kinase inhibitors are currently in different stages of clinical trials, and 8 compounds have already received FDA approvals. Initial strategies for the development of kinase inhibitors as anticancer drugs have focused on the selective inhibition of the target kinase represented as Gleevec. However, recent clinical trials of multi-targeted kinase inhibitors, such as sunitinib (Sutent®) have demonstrated that greatly enhanced antitumor activities, while still maintaining acceptable toxicity profiles [25]. Nevertheless, it is important to develop safer drugs by eliminating unnecessary kinase inhibitions, especially for non-oncology indications. Therefore, understanding of mechanism of actions for side effects observed with existing kinase inhibitors will be a great help in developing useful and safe drugs.

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Received: 07 May, 2008 **Revised: 30 June, 2008** **Accepted: 30 June, 2008**