Creating Chemical Diversity to Target Protein Kinases

B. Li[#], Y. Liu, T. Uno and N. Gray*

Department of Biological Chemistry, Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA

[#] *Current Address: RheoGene Inc., 2650 Eisenhower Ave., Norristown, PA 19403, USA*

Abstract: Protein kinases play crucial roles in regulating virtually every cellular process and are currently attracting tremendous interest as drug targets from the pharmaceutical industry. The major challenges facing the development of the potential kinase inhibitor drugs are: selectivity, physical properties (solubility, molecular weight), and pharmacological properties (bioavailability, half life, toxicity, etc.) This review focuses on how selective protein kinase inhibitors that target the ATP and allosteric binding sites are currently being identified and optimized.

Keywords: Kinase inhibitors, protein kinases, protein phosphorylation, signal transduction, kinase-targeted libraries, anti-cancer drugs, anti-inflammatory drugs.

INTRODUCTION

The protein kinases represent a large family of proteins which regulate a multitude of cellular processes, such as T-cell and B-cell activation, mitogenesis, platelet activation, neurotransmitter signaling, cell cycle control, growth control, oncogenesis, apoptosis, and transcriptional regulation [1-4]. All kinases catalyze the transfer of the terminal phosphate of ATP (adenosine triphosphate) to the hydroxyl of serine, threonine (S/T kinases) or tyrosine residues (tyrosine kinases) located on substrate proteins. This phosphorylation event can propagate a signal by a number of mechanisms: (1) Kinase Activation: kinases must frequently phosphorylate themselves (autophosphorylation) or become phosphorylated by "upstream" kinases on a portion of the catalytic domain called the "activation loop" which results in a conformation conducive to phosphate transfer, (2) Kinase Inactivation: the phosphorylation of c-terminal of c-Src by carboxyl-terminal Src kinase (CSK), for example, converts the kinase to an inactive form, (3) Substrate Recruitment: kinase phosphorylation can lead to the recruitment of activating or inhibiting partners that contain phosphate binding domains (e.g. Src homology domain 2 (SH-2), (4) Substrate Localization: phosphorylation can serve as a signal for altering cellular localization (e.g. phosphorylation of a nuclear localization motif (NLS) can alter the relative cytoplasmic/nuclear distribution), (5) Destruction Signal: phosphorylation can result in ubiquination and subsequent proteolysis. As kinase signaling lies at the heart of pivotal cellular decisions, kinase activity is typically strictly regulated. It should come as no surprise, therefore, that the loss of these regulatory mechanisms can lead to a number of pathological conditions. The most frequent mechanisms by which kinases become overactivated include: (1) Activating mutations: point mutations can result in "constitutively" activated kinases (e.g. mutation of phosphorylation sites on activation loop to acidic residues (D/E) that mimic phosphorylation). (2) Loss of inhibitory domains (e.g. viral Src (v-Src) vs. cellular Src (c-Src). (3) Fusion proteins: chromosomal translocations can result in creation of mutant proteins which are hyperactivated due to the presence of protein multimerization domains which result in excessive "transphosphorylation" or due to inappropriate cellular localization. (4) Loss of negative regulators: phosphorylation exists in equilibrium with dephosphorylation catalyzed by phosphatases (e.g. loss of PTEN phosphatase results in hyperphosphorylation of substrates of the AKT kinase). (5) Overexpression of kinase (e.g. as a result of chromosomal gene amplification or loss of transcriptional control). (6) Excessive stimulation by extracellular growth factors.

There are estimated to be 518 kinases encoded in the human genome, all of which have a highly conserved catalytic domain [5]. The catalytic domain consists of a bilobed structure with the catalytic residues situated in a deep hydrophobic cleft between the domains [6, 7]. The vast majority of small molecule kinase inhibitors developed to date target the ATP-binding site (Type I inhibitors), (Fig. (**1**)). This is not surprising as historically most inhibitors were discovered using biochemical screens of recombinant kinase catalytic domain at very low concentrations of ATP, where hydrophobic compounds are most likely to interact with the ATP-binding pocket. A serendipitous discovery allowed a second class of kinase inhibitor to be discovered that preferentially recognizes the "inactive" conformation of the kinase thereby preventing activation (Type II inhibitors). The binding site of Type II inhibitors extends from the ATP binding site to an adjacent hydrophobic pocket created by the "activation loop" being extended away from the conformation required to catalyze phosphate transfer. To date there appears to be only four series of Type II inhibitors disclosed in the public domain (STI571 (Gleevec®), BIRB-796 and its congeners, MLN518, BAY43-9006 and its congeners), three of which have been crystallographically proven (STI571, BIRB-796, BAY43-9006) [8-10]. Both STI571 and BIRB-796 are known to possess high kinase selectivity. A third class of kinase inhibitor/activator can be recognized as the one which modulate kinase activity by

*Address correspondence to this author at the Department of Biological Chemistry, Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA; E-mail: ngray@gnf.org

1386-2073/04 $45.00+.00 © 2004 Bentham Science Publishers Ltd.

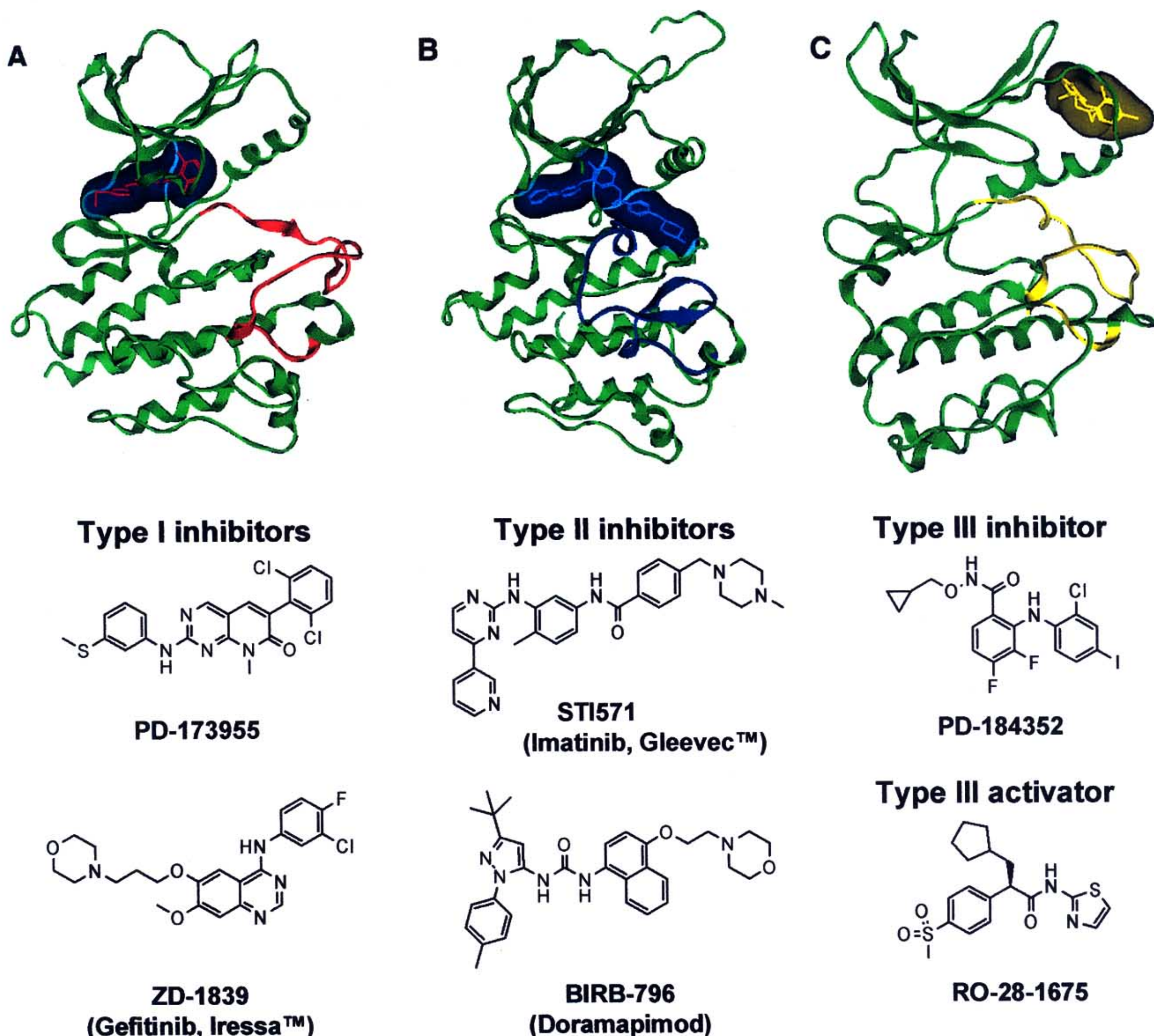

Fig. (1). Three general types of kinase inhibitor binding modes. Ribbon representation of the catalytic domain with (A) ATP-site inhibitor PD173955 bound to the active conformation of Abl, (B) STI571 bound to the inactive conformation of Abl, (C) PD184352 bound to an allosteric site on MEK kinase (a homology model). The conformation of the activation loop is shown in red (Abl, active conformation), blue (Abl, inactive conformation) and yellow (MEK, inactive conformation).

binding at sites, remote from the catalytic cleft. To date only few compounds have been disclosed that operate by this mechanism: the MEK inhibitor, PD184352 [11], and the glucokinase activator, RO-28-1675 [12]. It is noteworthy that PD184532 demonstrates a very impressive high selectivity profile against a panel of 28 kinases tested by Philip Cohen and his coworkers [13]. While ATP-competitive inhibitors are likely to be the most common kinase inhibitors, an increase in the utilization of cell-based and biochemical methods that specifically look for inhibitors of kinase activation will result in more Type II and III inhibitors being identified.

Given the increasing need for kinase inhibitors leads that can survive the demands of the drug development process, an overview of the different sources of new compounds is outlined below:

Screening of Historical Archives of Compounds Derived from Medicinal Chemistry Efforts: This method has proven to be successful in the past with the identification of the phenylamino pyrimidine motif that ultimately resulted in the development of Gleevec® [14] and the anilinoquinazoline scaffold that ultimately resulted in the development of Iressa® [15]. The major problem with this method is that as an increasing number of biochemical screens are performed with recombinant catalytic domains, the same hit compounds are identified time-and-time again. The utility of such historical archives can be revitalized through clever new screening strategies where allosteric inhibitors may be identified.

Screening of Compounds Derived from Historical Kinase Projects: Many groups have created screenable collections of all compounds derived from historical kinase-

directed medicinal chemistry programs. "Backscreening" these collections is frequently a tremendously efficient means of finding new uses for old scaffolds specially as the genomics era has resulted in more new kinase targets than truly distinct compound classes! The major problem with this approach is that often potent activity against the original kinase target must be engineered out whilst potency for the new target is dialed in. Screening these types of libraries often allows a unique facet of SAR to be uncovered with respect to a new target, but it does not often open avenues into a completely novel chemical space. Another problem is that many programs may be initiated based on a single scaffold that ultimately is demonstrated to have a fundamental liability.

The major deficiency of the aforementioned approaches is that they do not provide inspiration for the creation of truly new kinase inhibitory scaffolds. A number of approaches have been developed to create new potential kinase inhibitors.

Combinatorial Kinase Directed Libraries: This approach involves taking scaffolds that have proven to be kinase inhibitory or are very similar to known scaffolds and creating collections of compounds through scaffold and side chain variation (aka "soft focus library").

Creation of "Diversity" Oriented Libraries: "Diversity" oriented libraries are typically focused on chemistries that allow dense functionalization and often result in the creation of completely novel structures. Although these libraries are frequently called "natural product like, " these structures have not been subject to any sort of natural selection process and hence it is difficult to predict what biological activities they may have.

Creation of Amalgams or "Hybrids" of Previously Identified Inhibitors: This approach consists in creating new kinase inhibitors by grafting a portion of one inhibitor structure onto another in a manner that partially mimics the natural evolution of kinase sequences by genetic recombination and mutational events. These "hybrid" structures often possess physical properties and kinase selectivities completely unique from either parent structure.

Virtual Ligand Screening (VLS): This approach involves using computational methods (docking, energy minimization, scoring functions and so forth) to predict which compounds (virtual or real) will exhibit binding affinity to a particular kinase active site.

The following sections discuss the case studies of Type I and Type II inhibitors. This review is not intended to be comprehensive, and rather illustrates the utility of combinatorial approaches for the chemical development of kinase inhibitors. Thus, many examples are not included, and the interested reader is directed to several recent reviews [16-21].

TYPE I ATP-COMPETITIVE KINASE INHIBITORS

A. Purines as CDK Inhibitors

The cyclin-dependent kinases (CDKs) are serine/threonine kinases that play an important role in cell cycle progression

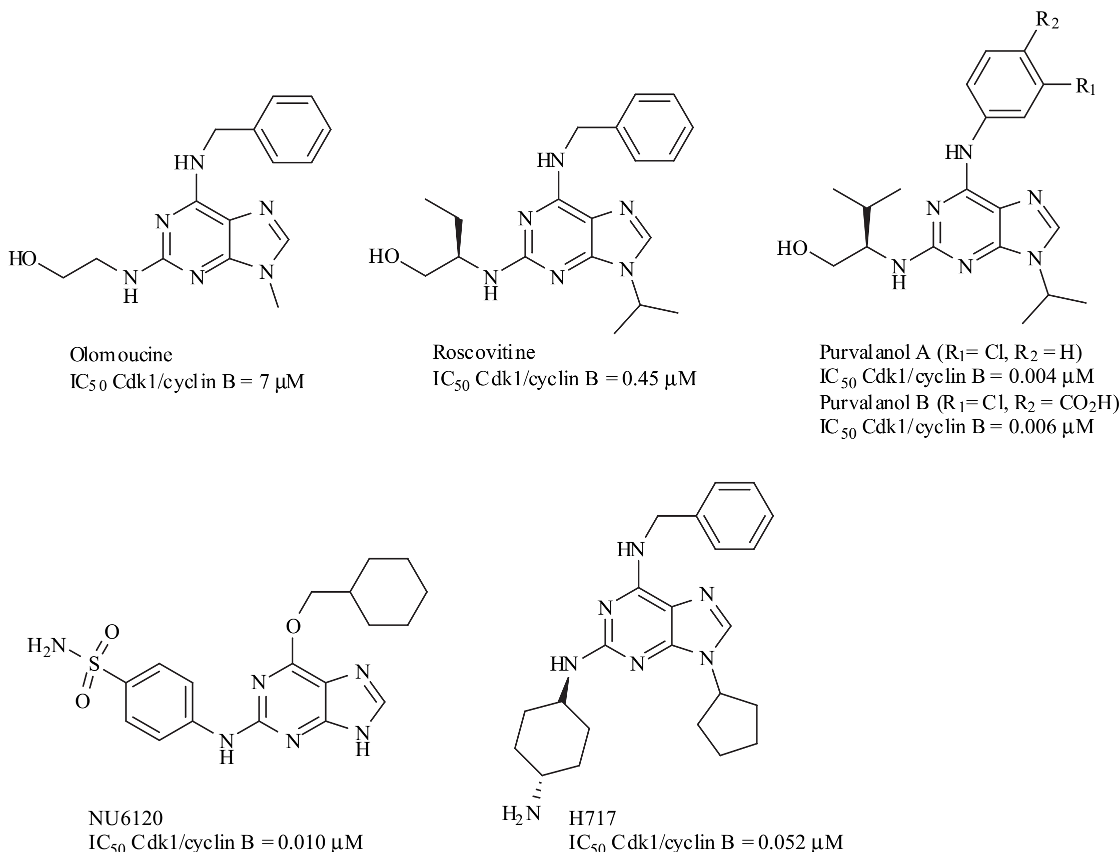

Fig. (2). Chemical structures of a selection of purine CDK inhibitors.

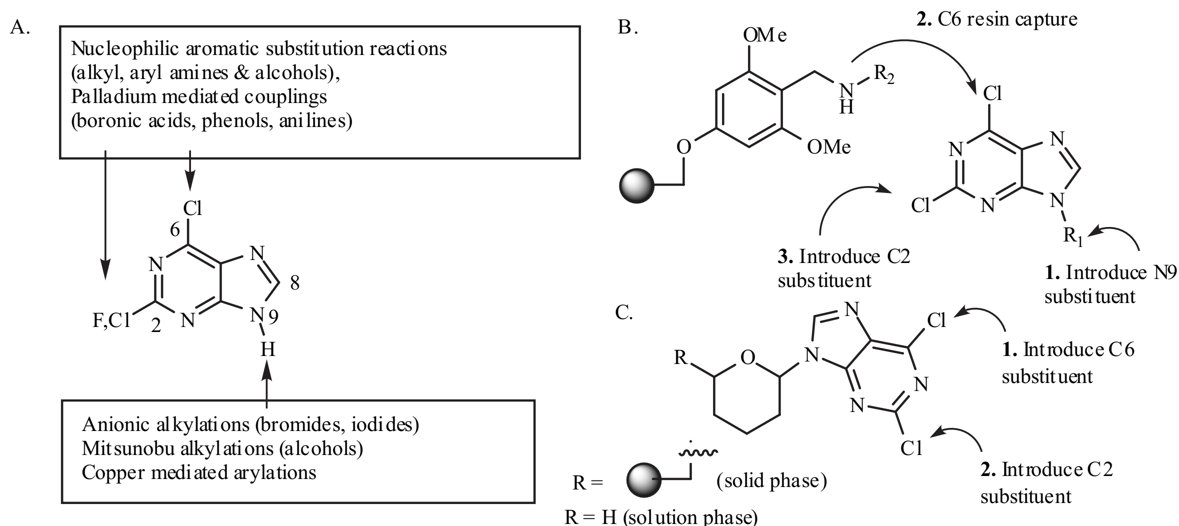

Fig. (3). Representative examples of synthetic strategies for preparing 2, 6, 9-trisubstituted purines. A. Chemistries used to introduce functionality to 2, 6, and 9 positions. B. & C. Solid and solution phase linkage and diversification schemes.

and provide a logical target for preventing cell proliferation [22]. The misregulation of CDKs has been detected in a variety of tumors [23]. A large number of CDK inhibitors have been discovered from natural products and synthetic libraries, including three compounds that have entered clinical trials: Roscovitine [20], flavopiridol [24] and UCN-01 [25]. Flavopiridol, however, was discontinued recently.

One well established class of kinase inhibitors are 2, 6, 9-trisubstituted purines. The founding member of this class was the natural product, Olomoucine [26], which was tested as a potential cyclin dependent kinase inhibitor due to its antimitotic activity, (Fig. (**2**)). The demonstration of Olomoucine's activity as an ATP-competitive inhibitor of Cdk1/cyclin B and the subsequent resolution of its co-complex structure with human Cdk2 catalytic domain [27], spawned a flurry of activity in academic and industrial labs [20]. Researchers developed a great variety of solution and solid-phase approaches for introducing diverse substituents to the 2, 6, and 9-positions of the purine ring. Screening of these compounds for their ability to inhibit starfish CDK1/cyclin B catalyzed phosphorylation of histone H1 resulted in the development of compounds such as Roscovitine [28, 29], Purvalanols [30], NU2058 [31, 32], and H717 [33], (Fig. (**3**)). These optimized structures possessed improved potency and selectivity for CDKs versus a variety of other kinase targets.

(*R*)-Roscovitine (CYC-202) is currently in phase II clinical trials for breast and non-small cell lung cancer and in phase I clinical trial for a hyperproliferative disorder of the kidney called glomerulonephritis [29, 34]. Interestingly NU6102 utilizes a rotated binding mode relative to the Roscovitine/Purvalanol-type compounds where the hinge

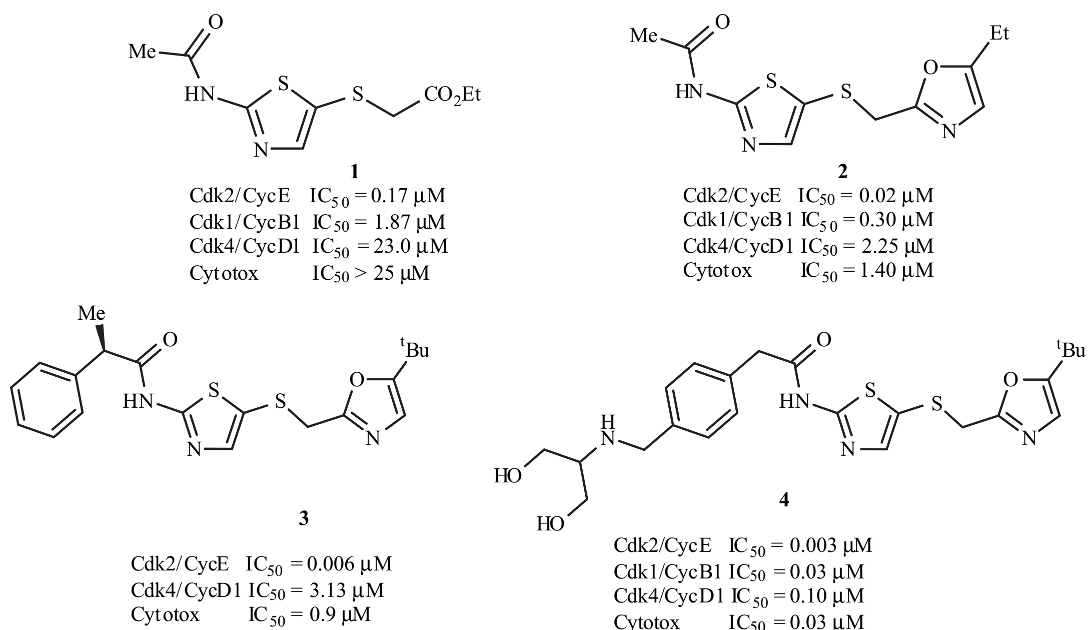

Fig. (4). The structures and activities of the aminothiazole Cdk2 inhibitors.

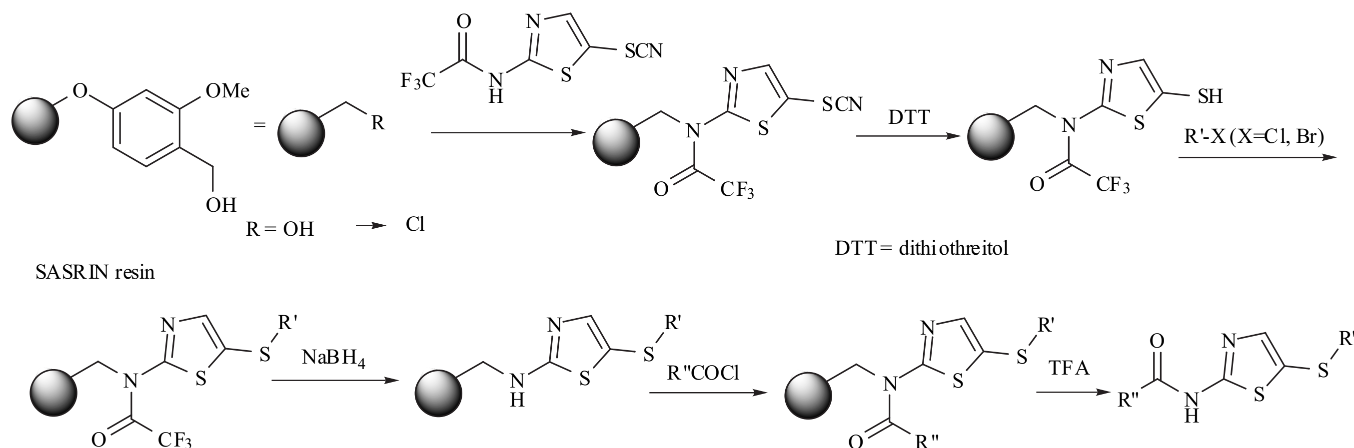

Fig. (5). The solid phase synthesis of the aminothiazole Cdk2 inhibitors.

region contacts are made through the donor-acceptor-donor motif represented by the C2 NH, N1, and N9 NH [20].

B. Aminothiazole as Cdk2 Inhibitors

High throughput screening at Bristol-Myers Squibb identified 2-acetamido-thiazolylthio acetic ester (**1**) as a relatively selective Cdk2/cyclinE inhibitor (IC_{50} = 0.17 μM) [35]. The small molecular size combined with potency, structural simplicity, and modular synthesis made the compound series of **1** a particularly interesting starting point for research, (Fig. (**4**)).

Despite its potent Cdk2 inhibitory activity, **1** lacks cellular activity (IC_{50} > 25 μM) most likely due to metabolism of the ester to the inactive carboxylic acid. A metabolically stable ester surrogate was sought to replace the ester in **1**, leading to key 5-ethyloxazole-substituted thiazole **2**. The oxazole (**2**), which is metabolically stable against esterases, maintained Cdk2 selectivity and displayed increased kinase inhibitory potency (**2** vs. **1**) as well as potent cellular activity with an IC_{50} of 1.4 μM. Combinatorial and parallel synthesis provided a rapid analysis of the structure-activity relationship (SAR) for these inhibitors against Cdk2, and over 100 analogs with IC_{50} values in the 1-10 nM range were rapidly obtained, (Fig. (**5**)).

Using solution phase parallel synthesis as well as individual compound synthesis, the authors rapidly prepared a series of thiazoles, which posses the nearly optimal 5-*tert*-butyloxazolylmethylthiol group in order to further explore the SAR of the 2-amino substitution. Among the analogs made in this effort, **3** provided the new lead structure for the further optimization. The x-ray structure of Cdk2 in complex with **3** revealed that the 2-amide side chain of **3** in the binding pocket of Cdk2 points toward the solvent exposed region. Introducing a polar amino alcohol group in this moiety led to a water soluble compound **4**, which is 10-fold and 30-fold selective for Cdk2/cyclinE vs. Cdk1/cyclinB1 and Cdk4/cyclinD1, respectively, (Fig. (**4**)). Compound **4** was 3-5 orders of magnitude more potent against Cdk2 than all other non-CDKs tested (protein kinase C (PKC) family members, IKK, Lck, EMT, ZAP70, and Her family and IGF-1 receptor enzymes). Compound **4** shows significant anti-tumor activity *in vivo* and possesses a favorable pharmacokinetic profile in mice.

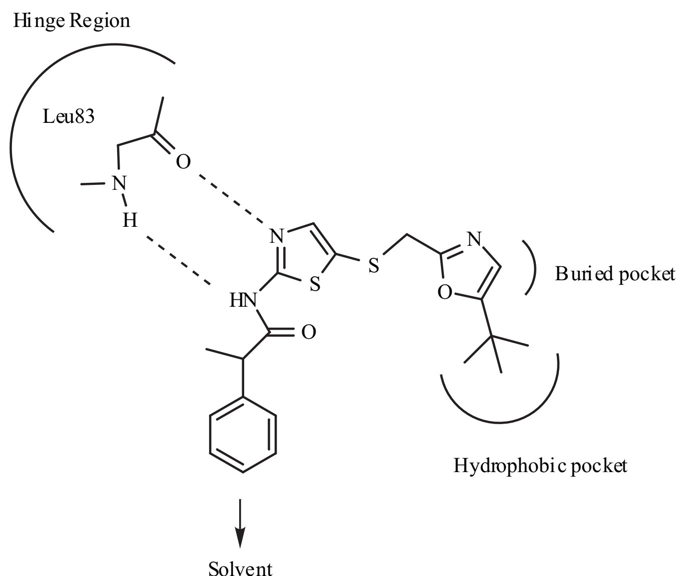

Fig. (6). Schematic binding mode of **3** with Cdk2 as determined by crystallography.

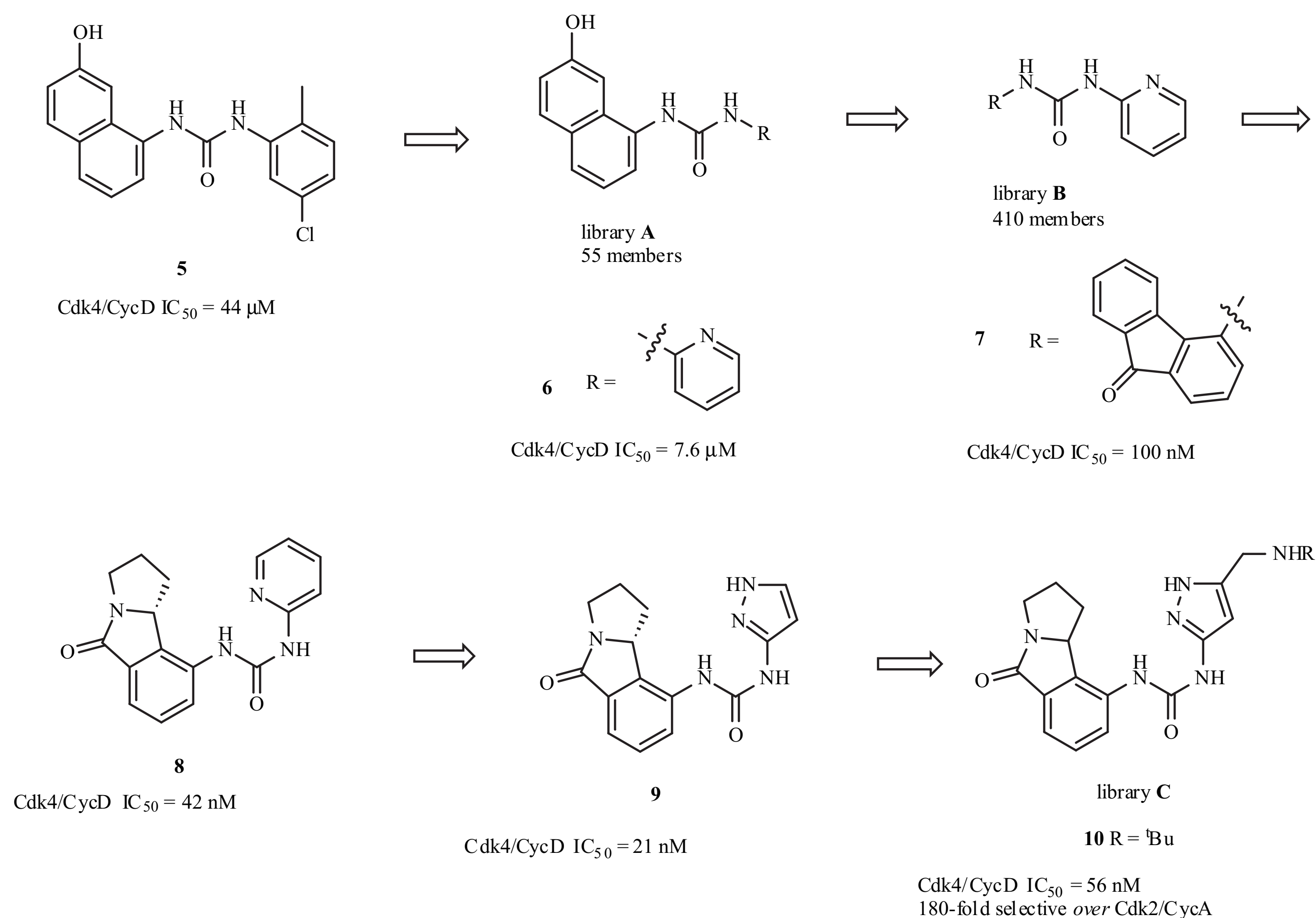

Fig. (7). Optimization of diaryl ureas against CDK4/Cyclin D.

C. Diaryl Ureas as Cdk4 Inhibitors

Cdk4 has also attracted interest as a target because unlike Cdk1 & 2, which control progression into the S and G2/M phases of the cell cycle, Cdk4/cyclin D activity is required to re-enter the cell cycle from G0 phase. Interestingly, the structure of the ATP site of Cdk4 is sufficiently different from those of Cdk1 & 2 that different spectrum of small molecules are active as inhibitors. To obtain highly selective and potent Cdk4 inhibitors, Honma and his coworkers at Banyu Pharmaceuticals performed a structure-based design consisting of the following two steps: (1) lead generation by rational design using a Cdk4 homology model derived from the x-ray structure of Cdk2 and (2), enhancement of selectivity for Cdk4 versus Cdk1/2 and the other kinases based on the binding modes and structural differences between Cdk4 and other kinases [36, 37]. As the first step to generate new scaffold candidates on the basis of this homology model, they applied a new *de novo* design strategy which combined the *de novo* design program LEGEND with their in-house structure selection supporting system SEEDS. This analysis resulted in the identification of four distinct classes of inhibitors which possessed IC_{50}s between 15−500 μM for Cdk4/cyclinD: diarylureas, cyclic ureas and thioureas, 4-aryl-2-aminopyridines and triazines, and pteridines. Among them the diaryl urea class compounds showed 2-digit μM potency, for example, **5** exhibited a Cdk4/cyclinD activity of 44 μM, (Fig. (**7**)).

In order to rapidly study the SAR for Cdk4 inhibition, a series of small libraries were prepared by solution phase synthesis, (Fig. (**7**)). First, a 55-membered library **A** was synthesized where the 7-hydroxynaphthyl group was held constant. Screening of this library revealed that, while aliphatic groups exhibit diminished activity, compounds with 2-pyridyl group (**6**) and 2-thiazolyl group show 7.6 μM and 23 μM activity, respectively. The position of the nitrogen in the aromatic ring seemed important, for compounds with 3-pyridyl or 4-pyridyl showed poor activity. Next, a 410 member library **B** was prepared with the 2-pyridyl group held invariant. Screening of this library allowed **7** to be identified as a 100 nM Cdk4 inhibitor. Further optimization of the fluorenonyl group led to **8** (IC_{50} = 42 nM). The authors validated the proposed binding mode of diarylurea inhibitors by solving the x-ray structure of the Cdk2-**7** complex.

Compound **8** showed good Cdk4 inhibitory activity and moderate selectivity (55-fold to > 480-fold) over other kinases. However, **8** inhibited Cdk1 and Cdk2 in addition to Cdk4/6 with comparable IC_{50} values, which was not surprising due to the method used to obtain these lead compounds and the similarity among the CDK family. As their second step of optimization to enhance the selectivity, they initially identified specific amino acid residues around the ATP binding pocket of Cdk4 by comparing the amino acid sequences of 390 representative kinases. This analysis

allowed eight residues which are different between Cdk1/2 and 4 to be identified. The location of these amino acids in relation to the predicted binding mode of **8** was used to design a chemical library. Specifically, five of the eight residues are distributed on one side of the ATP binding pocket, which is the binding site of the endogenous CDK-inhibitor p16. The binding mode of **8** indicated the pyridine ring of **8** is directed towards the amino acid residues in the p16 binding region. Compound **9** (Cdk4/cyclinD IC_{50} = 21 nM) with a 5-member-ring pyrazole group was found to be a useful lead in addition to **8**. The binding modes predicted the 5-position of the pyrazole ring in **9** to be the most appropriate position and direction to the p16 binding region. To design suitable substituents at the 5 position, the *de novo* design programs, LUDI and LeapFrog, were employed, and 64 member library **C** was constructed. A selective compound **10** (180-fold selective *vs.* Cdk2/cyclinA) was identified. On the basis of the insights obtained from the SAR of library **C**, several compounds were designed, and one of them, **11**, exhibited a very high selectivity over Cdk1/cyclinB and Cdk2/cyclinA, (Fig. (**8**)). Compound **11** also showed greater than 7000-folds selectivity over other Ser/Thr kinases (PKA, PKC, PKBα, CaMK II, p38α, ERK1, and MEK1) and Tyr kinases (Src, Lck, Flt-1, ZAP70, EGFR, FGFR1, and PDGFRβ).

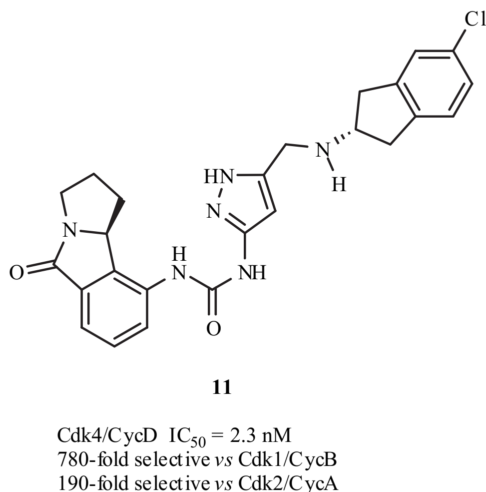

Fig. (8). Activity and selectivity of CDK4 inhibitor **11**.

A docking study of **7** with CDK4 predicts that an intramolecular H-bond constrains the urea to a *cis* conformation allowing the carbonyl oxygen and urea amino to form a pair of H-bonds to the hinge region, (Fig. (**9**)). The fluorenone carbonyl oxygen is predicted to form a hydrogen bond with the ε-NH_2 group of Lys35 in the hydrophobic region. The X-ray analysis of the Cdk2-**8** complex is consistent with the binding mode of **7** with Cdk4. The binding mode of the most optimal compound (**11**) with Cdk4 appears to be similar, but the 5-chloroindan-2-ylaminomethyl group enhances the hydrophobic interaction with Cdk4.

The development of diaryl ureas by Honma *et al.* demonstrates a number of important principles: (1) The use of multiple iterative rounds of synthesis and screening of small focused sublibraries such that substituents that enhance activity can be incorporated in the next synthetic iteration, (2) the use of molecular modeling to rationalize improvements in potency and selectivity such that new library substituents can be chosen that conserve features of previously identified compounds (i.e. replacing fluorenone by pyrrolo[2, 1-a]isoindolone).

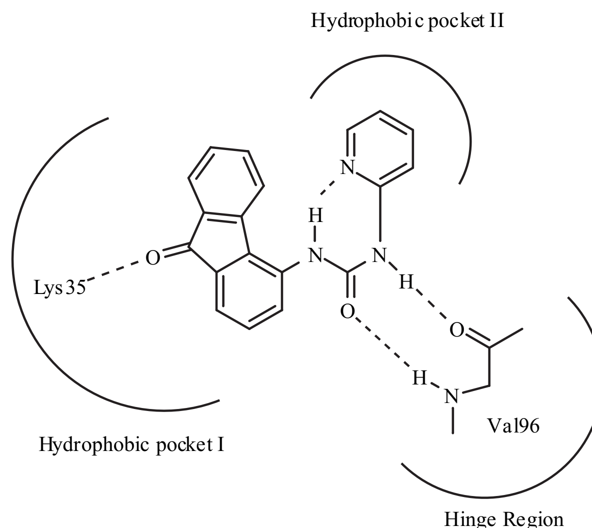

Fig. (9). Binding mode of **7** with Cdk4.

D. 4-Acylamino-1, 3-Thiazoles as Potential Cdk5 Inhibitors

Cyclin-dependent kinase 5 (Cdk5), a serine/threonine kinase, is required for proper development of the mammalian central nervous system [38, 39]. Deregulation of Cdk5 has been implicated in pathology of Alzheimer's disease, as a kinase potentially involved in the hyperphosphorylation of Tau [40]. Therefore, the inhibition of Cdk5 may be useful for the treatment and/or prevention of Alzheimer's Disease [40]. Cdk5 through its ability to phosphorylate dopamine and cyclic AMP-regulated phosphoprotein (DARP-32) is able to dampen dopaminergic signaling suggesting that Cdk5 inhibitors might be useful for the treatment of some forms of depression or Parkinsons [41, 42].

Pharmacia researchers identified a lead inhibitor **12** of Cdk5/p25 (K_i = 0.5−2.0 μM) by broad screening of its compound collection [43]. It also showed modest selectivity versus the homologous kinase complex Cdk2/cyclin A (K_i = 10−20 μM), (Fig. (**10**)). To circumvent contamination by numerous impurities formed as a result of solution phase synthesis, a catch-and-release approach was developed for the parallel synthesis of two-dimensional combinatorial libraries of 4-acylamino-1, 3-thiazoles for optimization of the activity and selectivity of this series, (Fig. (**11**)).

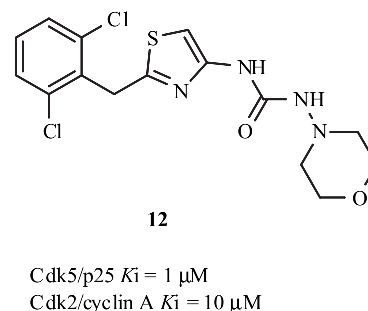

Fig. (10). Structure and CDK activity of screening lead **12**.

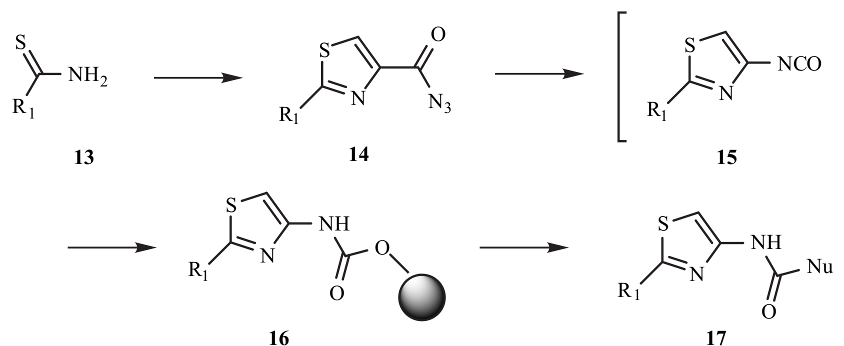

Fig. (11). Solid phase synthesis of 4-acylamino-1, 3-thiazoles using a catch-and-release approach.

The acylazides (**14**), prepared from thioamides **13**, underwent thermolysis to generate the intermediate isocyanates (**15**), which were reacted with an oxime modified resin [polystyrene-C(4–NO_2–Ph)=NOH] to afford thiazolecarbonate resin **16**. The impurities were removed by washing repeatedly with dichloromethane and methanol. The desired thiazoles (**17**) were released by treating the washed resins with nucleophiles in the presence of triethylamine. Purification of the crude products was accomplished by combination of scavenger resins and solid-liquid extraction techniques. A combination of 22 thioamides, 56 hydrazines and 9 alkoxyamines were used for preparing several two-dimensional libraries (50–100 compounds each- not all possible combinations were prepared). Most of the library products showed more than 80% purity as determined by HPLC/MS and gave average yields of 70%.

The SAR revealed that only analogs derived from 1, 1-disubstituted hydrazines retained activity. The library members with monosubstituted and 1, 2-disubstituted hydrazine elements were inactive. With respect to the 2-substituent of the thiazole ring, no substituent improved activity and selectivity versus Cdk2/cyclin A relative to the 2, 6-dichlorobenzyl moiety present in the original lead. Therefore the lead (**12**) remained to be the best among the hydrazine series after the library synthesis. However, replacing the hydrazine moiety of **12** with alkoxyamines afforded the compounds with remarkably good activity and selectivity, leading to the identification of the optimum compound (**18**), which exhibits submicromolar activity for Cdk5/p25 and over 100-fold selectivity versus Cdk2/cyclin A, (Fig. (**12**)).

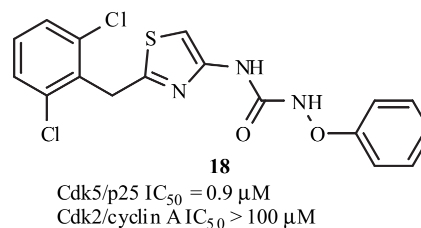

Fig. (12). Structure and CDK activity of improved compound **32**.

E. Oxindoles as Janus Kinase 3 Inhibitors

Inhibitors of Janus Kinase 3 (JAK 3) have shown activity as immunosuppressant agents [44] and have been proposed as potential drugs for transplantation and diabetes. The initial screen of the kinase-biased set at Aventis yielded oxindole **19** as hit of micromolar potency, (Fig. (**13**)) [45].

To further optimize the inhibitor, a homology model was generated on the basis of the cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA) and the insulin receptor tyrosine kinase (IRK). From the homology models, it is predicted that the oxindole forms a hydrogen bond pair with carbonyl of Glu903 and NH of Leu905. Analysis of the docked inhibitor indicated that the pyrrole ring projects outward in the direction of solvent, although the ring itself lies within the ATP pocket. Variation at this region in the library was intended to explore this space fully, to identify possible interaction sites both inside the pocket and outside on the lip, but also to explore the potential for the attachment of groups to give

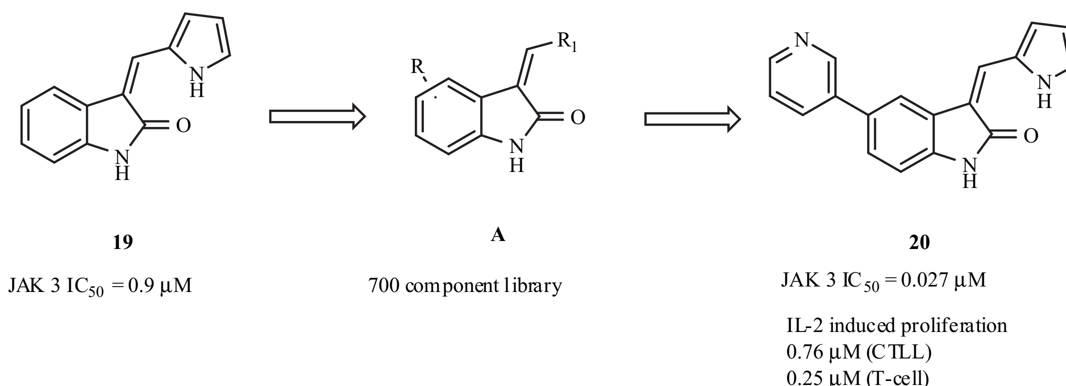

Fig. (13). Optimization of JAK3 activity of oxindoles.

better pharmacological properties without necessarily interacting with the kinase. In contrast, it could be seen that the vector of the CH atom at the 5-position of the oxindoles core pointed into an unfilled region of the active site. Thus, to explore both the pyrrole position and 5-position of oxindoles, a two-dimensional library was prepared in a solution phase parallel synthesis fashion by condensation of oxindole cores with proprietary and commercially available aldehydes. The final products were isolated by precipitation, and those that exhibited purities greater than 85% by LC/MS were accepted as the library components. The final array consisted of 700 inhibitors, which were then screened against the JAK 3 kinase domain.

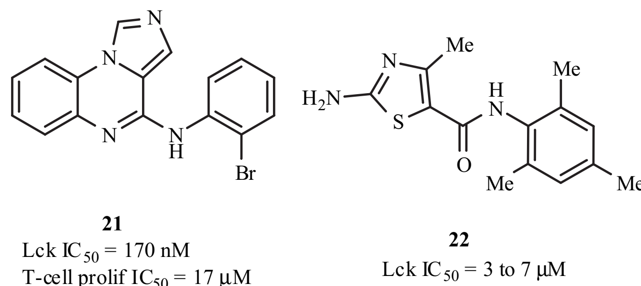

Fig. (14). BMS' high throughput screening leads **21** and **22** for Lck.

Compound **20** with 3-pyridyl at 5-position gave 27 nM JAK 3 potency. Electron-donating aromatics on the other side of the double bond increased potency, but the most potent one being pyrrole. Compound **20** inhibited IL-2 induced cell proliferation (IC_{50} = 0.76 μM for mouse CTLL cell, and IC_{50} = 0.25 μM for human T-cell). Compound **20** also demonstrated an efficacy comparable to dexamethasone in the ear edema model upon topical administration.

F. Imidazoquinoxalines as Lck Inhibitors

Lymphocyte-specific protein-tyrosine kinase (Lck), a member of the Src-family of non-receptor tyrosine kinases, plays an essential role in T-cell development and activation [2, 46-49]. Lck initiates T-cell antigen receptor (TCR) signaling ultimately resulting in cytokine production and T-cell activation by phosphorylating Immunoreceptor Tyrosine Activation Motif (ITAM), Syk, and ZAP-70 tyrosine kinases [4]. Inhibition of Lck has been proposed as a potential method for treating autoimmune and inflammatory disorders such as rheumatoid arthritis, multiple sclerosis, transplant rejection and delayed hypersensitivity reactions [50-54].

High throughput screening at Bristol Myers Squibb (BMS) identified 1, 5-imidazoquinoxaline **21** as a relative potent and selective Lck inhibitor lead (Lck IC_{50} = 170 nM, T-cell proliferation IC_{50} = 17 μM) [55] and thiazole **22** as a moderate potency Lck inhibitor lead (Lck IC_{50} = 3.2 μM), (Fig. (**14**)) [56]. These structures are interesting because they represent novel tyrosine kinase inhibitor motifs.

In order to explore the SAR of the 2-position of lead **21**, approximately 160 compounds were prepared by parallel solution phase synthesis, (Fig. (**15**)). Replacement of the 2-bromoaniline with aliphatic and aromatic amines resulted in a substantial loss of activity against Lck. Both oxygen and sulfur linkers were significantly less potent than the amine linker. A biaryl analog and *N*-methyl analog both displayed poor activity suggesting that the aniline N-H is important for activity. Optimal substituents were 2, 6-disubstituted anilines, and the compound containing 2-chloro-6-methyl aniline (**23**) showed 9 nM Lck inhibitory activity. Next substitutions on the imidazoquinoxaline were explored and optimized by an automated solid phase synthetic approach, (Fig. (**16**)). Substitution at the 2- or 4-position on the imidazole ring with small alkyl group is tolerated, and electron-donating groups on the fused phenyl ring enhance the potency. Finally, the optimization of **21** resulted in the discovery of **24** with potent enzymatic activity (Lck IC_{50} = 2 nM) and good T-cell proliferation inhibition (IC_{50} = 0.67 μM).

The binding model of **21** based on the published coordinates of activated Lck kinase domain is shown in (Fig. (**17**)). The phenyl ring is twisted out of the plane of the tricyclic ring system and fits into a hydrophobic pocket. This hydrophobic pocket favors small size, electron-withdrawing, di-ortho substituted phenyl ring, which is consistent with the high potency of **23**. The nitrogen of the imidazole ring and the NH of the aniline are hydrogen bonded to Met319 and Thr316 at the hinge region, respectively.

In the subsequent paper, the BMS researchers further optimized the cellular activity of **23** [57]. Thus, using a

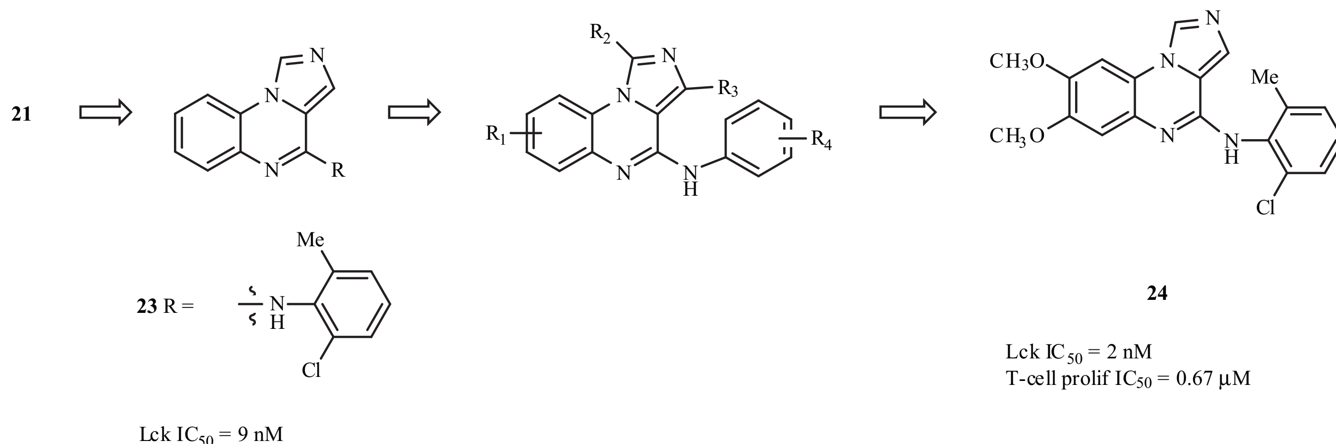

Fig. (15). Optimization of imidazoquinoxaline-based Lck kinase inhibitors.

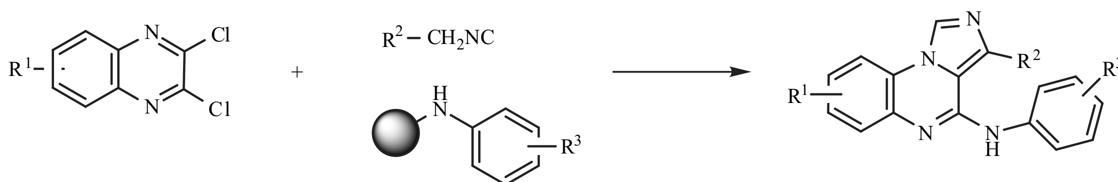

Fig. (16). Solid phase synthesis of imidazoquinoxalines.

similar approach of parallel synthesis, compounds with significant improved cellular activity (IC_{50} <200 nM) were identified by incorporating polar and weakly basic amine-bearing side chains into the 7-position of the fused phenyl ring of the imidazoquinoxaline core. The best two compounds, **24** and **25**, are shown in (Fig. (**18**)).

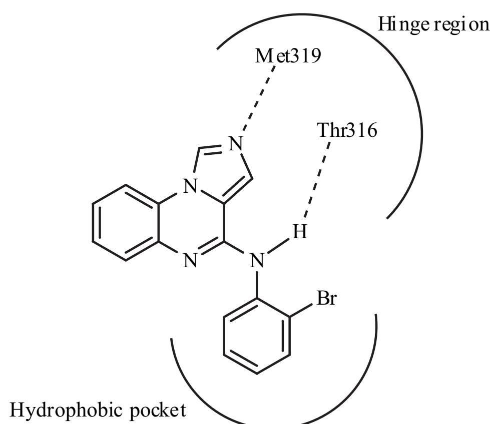

Fig. (17). Binding model of **21** at the ATP binding site of Lck kinase.

The optimization of thiazole carboxamide **22** started with examining the effects of modifications to the thiazole core [56]. It was quickly discovered that benzothiazole **26** was an order of magnitude more potent than **22**, (Fig. (**19**)). Thus, the researchers at BMS optimized both thiazole and benzothiazole derivatives [58, 59]. The SAR of the aniline ring of **22** and **26** parallels to that of the imidazoquinoxaline class Lck inhibitors (see above), with 2-chloro-6-methylaniline being optimal. A large number of carboxamides **28** and **31** and urea derivatives **29** and **32** were prepared using multiple rounds of solution phase parallel synthesis, (Fig. (**20**)).

For the amide series, the cyclopropylamides were identified to be the most potent Lck inhibitors: **33** and **34**, (Fig. (**21**)). Modification of the C2 amino group as a urea also led to some very potent Lck inhibitors for both the thiazole class and the benzothiazole class. In fact, urea **35** was one of the most potent inhibitors discovered in these studies. When tested against a panel of kinases, thiazole **33** exhibits excellent selectivity for the receptor tyrosine kinases and serine/threonine kinases, but no significant selectivity was observed for the seven other Src family members. In contrast, benzothiazoles **35** showed moderate to good selectivity for other member of the Src family kinases. In addition, compound **35** exhibited at least 6000-fold selectivity over Cdk2, IGF1R, KDR, and p38 kinases, (Fig. (**21**)). They all showed good cellular activity.

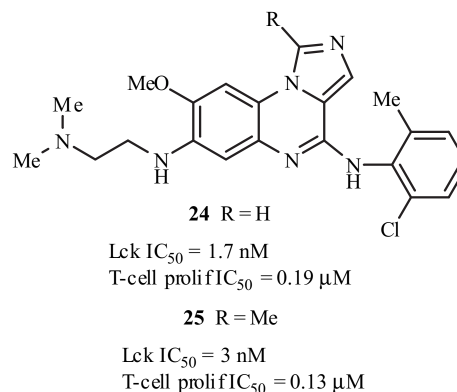

Fig. (18). The optimized imidazoquinoxaline-based Lck inhibitors.

Additional studies were performed with the goal of discovering suitable replacements for the urea moiety of **35** [59]. Thus, a wide variety of analogs were prepared using the parallel solution phase synthesis from key intermediate **36**, leading to the discovery that 2-pyrimidyl and 4-pyrimidyl analogs possess excellent potency, (Fig. (**22**)). Further optimization culminated in 2-aminopyridyl analog, BMS-350751 (**37**), and 2-aminopyrimidinyl analog, BMS-358233 (**38**), as highly potent Lck inhibitors *in vitro* with excellent activity in a T-cell proliferation assay, (Fig. (**23**)).

The binding models predict that **35** and **37** bind in a similar extended conformation to the ATP binding site, (Fig. (**24**)). The 2, 6-disubstituted aniline fits into a narrow and angular hydrophobic pocket.

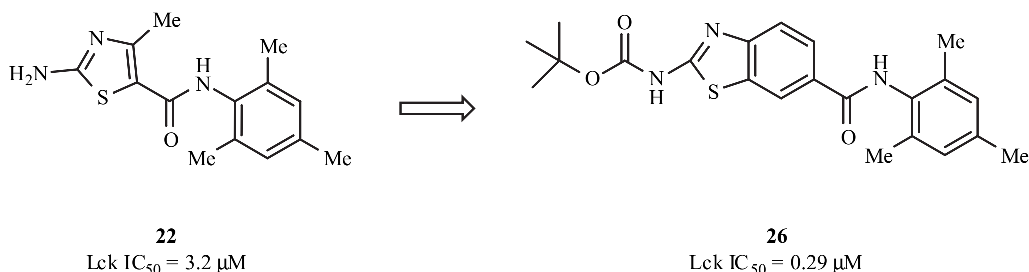

Fig. (19). Exploration of the core structure lead to benzothiazole scaffold.

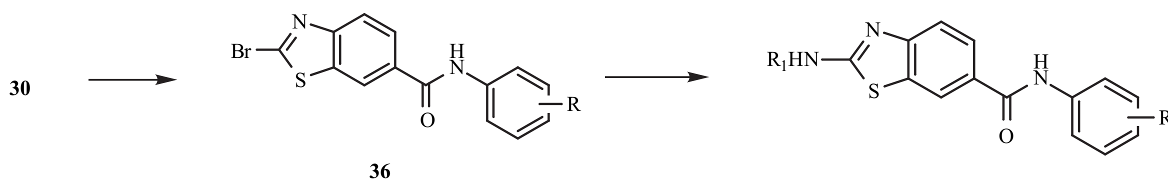

Fig. (22). Additional study of **35** by parallel solution phase synthesis.

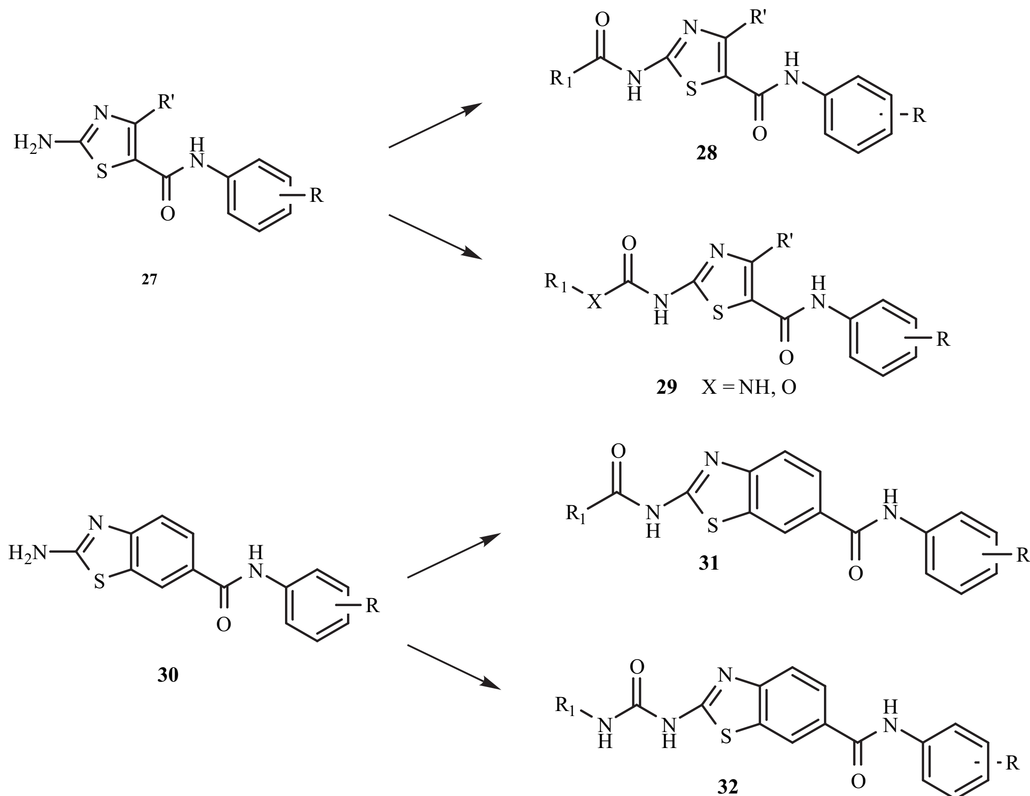

Fig. (20). Solution phase parallel synthesis of 2-amino group analogs of **27** and **30**.

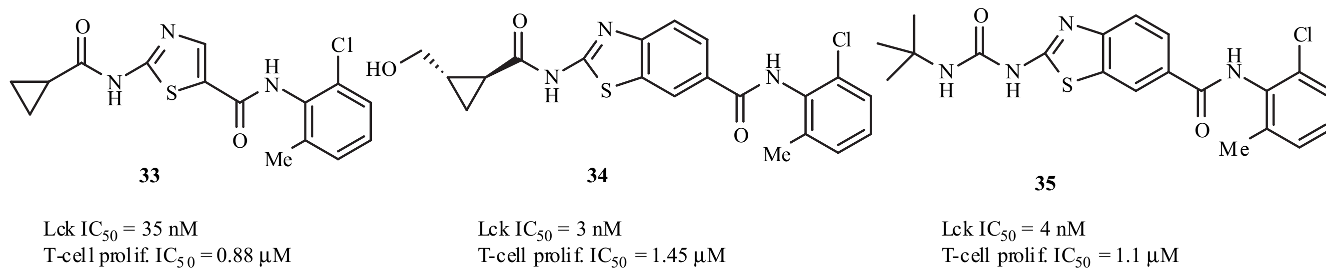

Fig. (21). Lck activities and T-cell activities of **33**, **34** and **35**.

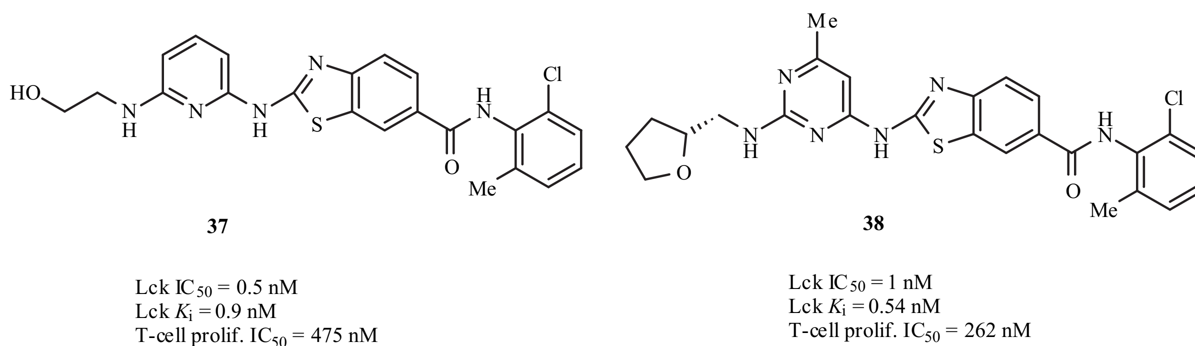

Fig. (23). Lck inhibitory activity of benzothiazoles **37** and **38**.

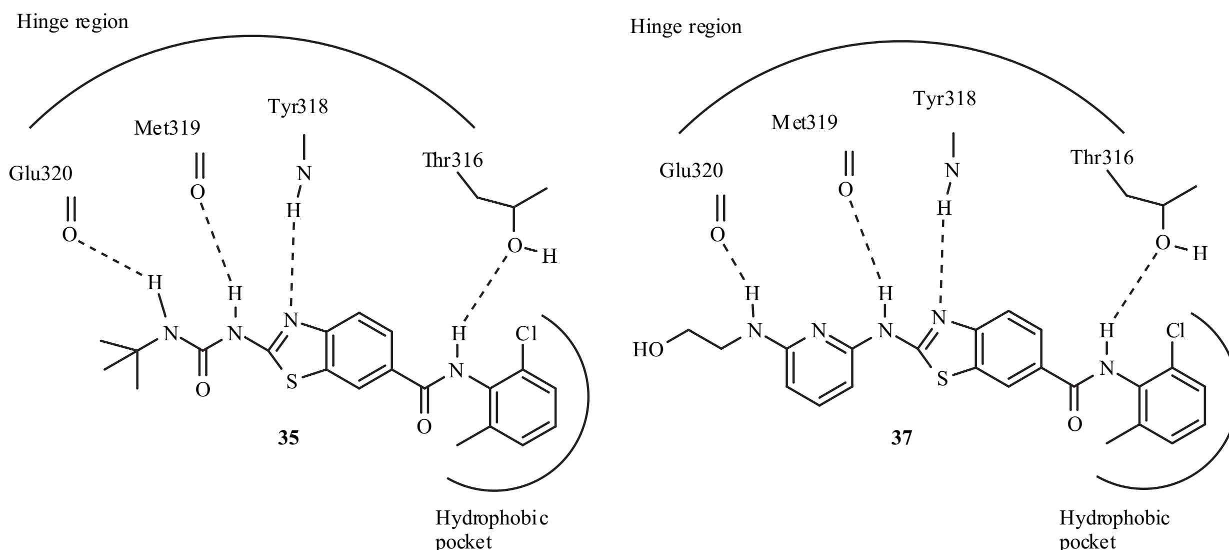

Fig. (24). Proposed binding mode of **35** and **37** at the ATP site of Lck.

G. Imidazole-Based p38 MAP Kinase Inhibitors

The p38 MAP kinases, a family of serine/threonine protein kinase, play crucial roles in transducing extracellular stress signals [60]. Pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1-β (IL-1β) are believed to be causal agents of chronic inflammatory diseases such as rheumatoid arthritis [61, 62]. The synthesis of TNF-α and IL-1β is regulated by MAPK p38 [63, 64]. Several p38 inhibitors have advanced into human clinical trials including two imidazole-based inhibitors, SB242235 (**39**) [65] and RWJ-67657 (**40**) [66], a pyrazole-based inhibitor RO3201195 (**41**) [67], a urea based inhibitor BIRB-796 (**42**) [68-70] and pyrimido[4, 5-d]pyrimidinone VX-745 (**43**) [71], (Fig. (**25**)).

Researchers at Aventis reported the development of RPR200765A (**46**) [72] and RPR238677 (**47**) [73] using a solution phase and a solid-phase combinatorial approach, respectively. In the discovery of RPR200765A, from initial lead RPR132331 (**44**), a weak inhibitor of TNF-α (EC_{50} = 800 nM), seven libraries totaling over 200 compounds were synthesized using parallel synthesis and 190 were accepted for the screening in four months, (Fig. (**26**)). The libraries were carefully designed with respect to H-bonding, charge, hydrophobic and aromatic interactions. In addition, molecular weight less than 550 and cLogP less than 5 were applied to achieve good oral bioavailability. The screening strategy for compound progression includes: cellular activity, *in vivo* activity, bioavailability, disease-modifying activity, kinase selectivity, and physicochemical properties for formulation. Also included in the screening strategy were *in vitro* and *in vivo* toxicity such as Cytochrome p450 (CYP) 1A1 induction, mutagenicity, and guinea pig toxicity, as toxicity was an issue with this class of compounds.

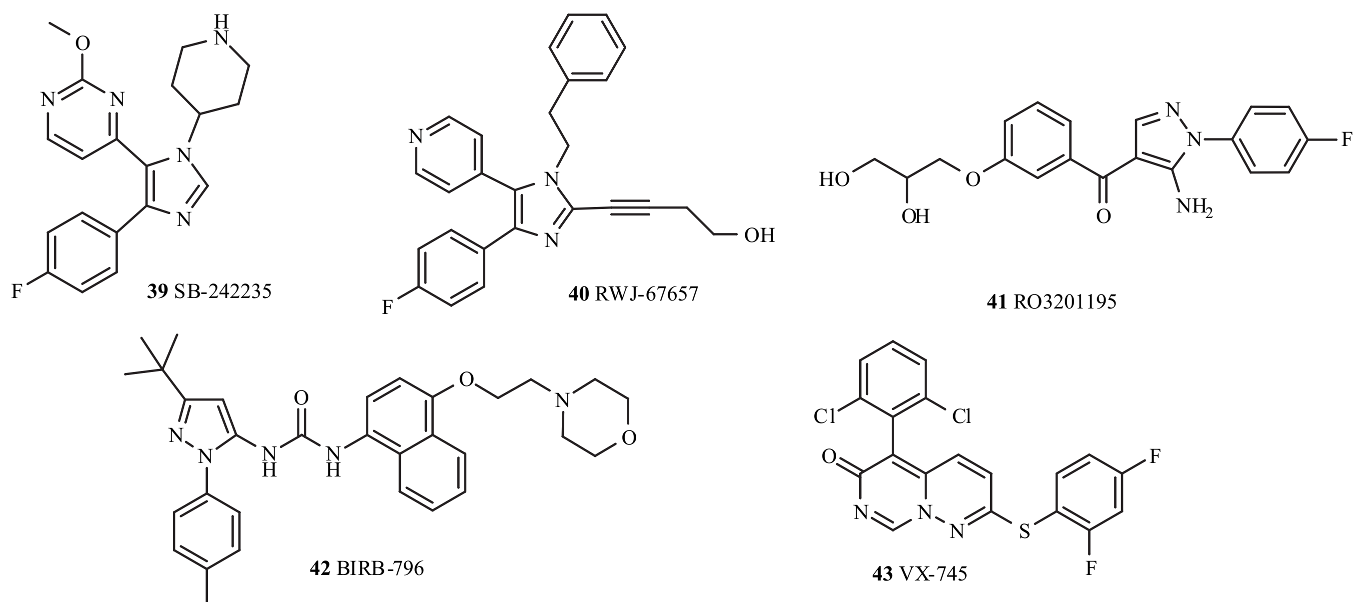

Fig. (25). p38 MAP kinase inhibitors in clinical trials.

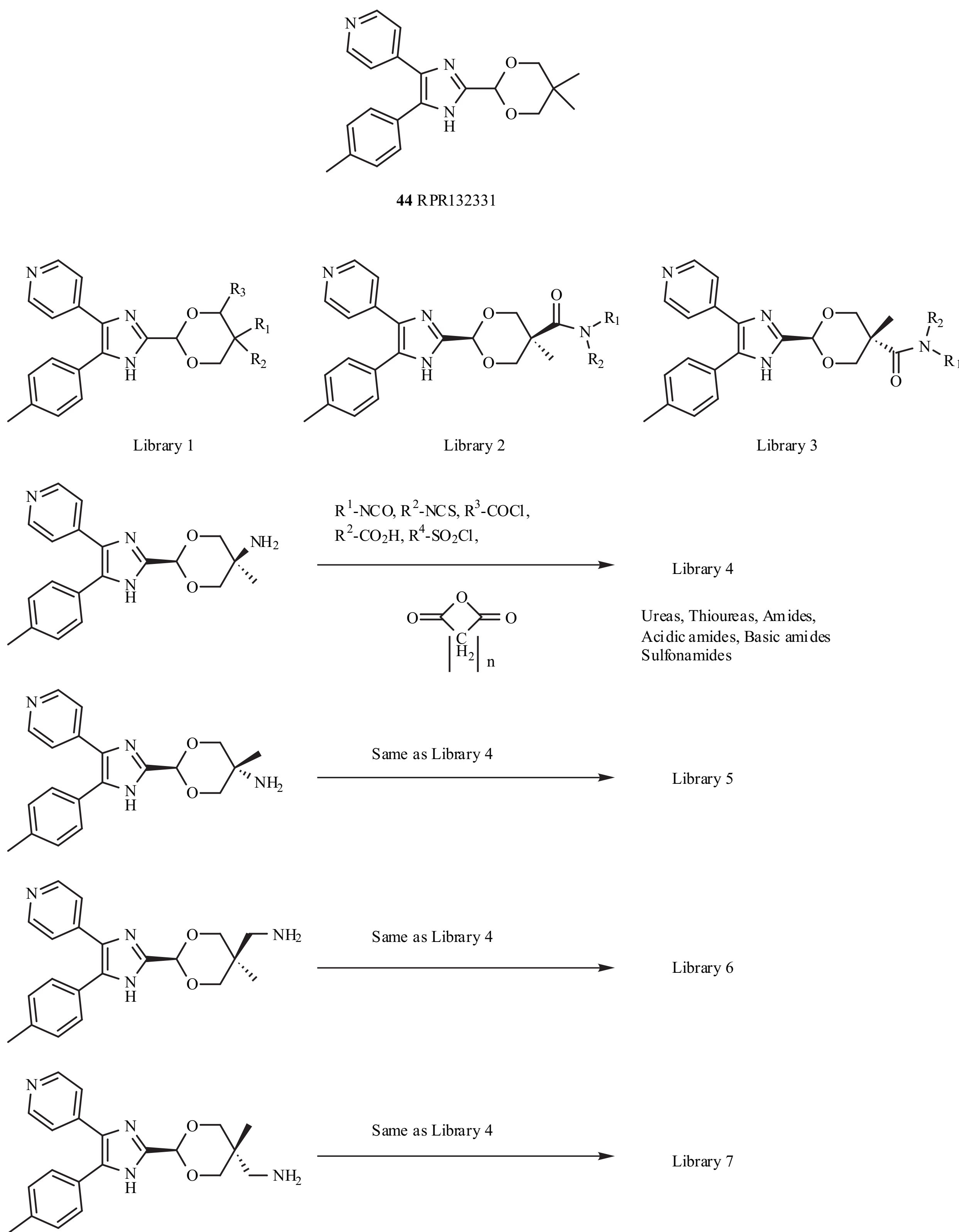

Fig. (26). Libraries of imidazole-based p38 MAP kinase inhibitors by parallel solution phase synthesis.

Out of 190 compounds, 24 passed the first cellular functional assay (TNFα release from monocytes, EC_{50} < 200nM), 12 compounds showed *in vivo* activity (TNFα release in the mouse, ED_{50} < 30 mg/kg) for the secondary screening, and four compounds were non-inducers of CYP1A1 in the third test. Two mesylate salts, RPR200765A and RPR201227A, showed excellent bioavailability with high blood levels in the rat. RPR 201227A (**45**) was abandoned because of the moderate activity to ACAT (acyl-CoA: cholesterol *O*-acyl transferase)

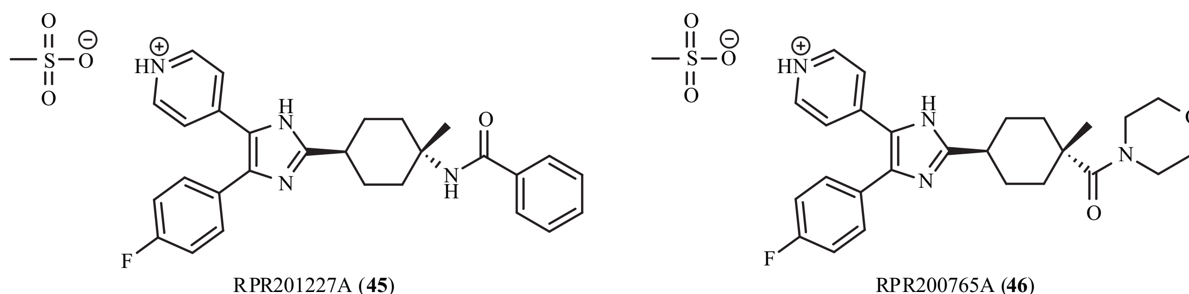

Fig. (27). Structure of RPR 201227A (**45**) and RPR200765A (**46**).

and high toxicity to guinea pigs (100 mg/kg/day) while RPR200765A (**46**) was accepted as a development candidate for the potential treatment of rheumatoid arthritis due to the excellent oral bioavailability (50%), disease modifying activity, the absence of toxicity indicators and its excellent physical properties, (Fig. (**27**)).

As a follow-up study, solid-phase combinatorial synthesis of pyrimidinyl-imidazole-based library (570 member) with two points of structural modification resulted in the rapid identification of a potential drug candidate RPR238677 (**47**), (Fig. (**28**)) [73]. In this work, the researchers at Aventis developed the Monte Carlo Monomer Selection (MCMS) algorithm, which utilized synthetic constraints and absorption/bioavailability requirements to constrain the library to maximize the combinatorial synthetic efficiency and the bioavailability of the final compounds. Key intermediate **48** and its diastereomer were prepared in solution and loaded onto a Merrifield thiol resin for elaboration of the right hand amide moiety. The desired products were released from the resin upon treatment with the second diversity element amines, followed by purification using isocyanate resin as scavenger, (Fig. (**29**)). The cleavage procedure from the solid support yielded a 570-member library; analysis of these showed that 85% of the compounds were of greater than 85% purity, with 90% of these being of 100% purity using a parallel LC-MS in conjunction with evaporative light scattering (ELS) detection.

Following parallel analyses of the compounds in a primary functional assay, the CaCo-2 monolayer assay, and in an *in vitro* metabolic stability study, an assessment of the compound bioavailability was carried out in a combi-PK cassette-dosing experiment. The MCMS designed objective appears to have been born out with 80% of the compounds exhibiting a high absorption as determined by the CaCo-2 monolayer assay. Within the designed library, 85% of the compounds surpassed the activity of the reference compound RPR200765A (**46**) for inhibition of p38 kinase activity. Out of 56 compounds selected for a combi-pharmacokinetic (PK) cassette-dosing experiment, ten compounds were identified as worthy of *in vivo* efficacy studies. These compounds were relatively structurally diverse and possessed an improved *in vitro* potency over RPR200765A (**46**) and good Cp max values and AUCs in the rat. Interestingly, there is no obvious structure-activity relationship (SAR) that can be discerned from these ten compounds, demonstrating the strength of the combinatorial approach combined with the rational design strategy. These compounds could not have been obtained as rapidly following a traditional sequential approach.

TYPE II INHIBITORS

A. Diaryl Urea as p38 MAP Inhibitor

Type II inhibitors preferentially recognize the "inactive" conformation of the kinase thereby preventing activation. The binding site of type II inhibitors extends from the ATP

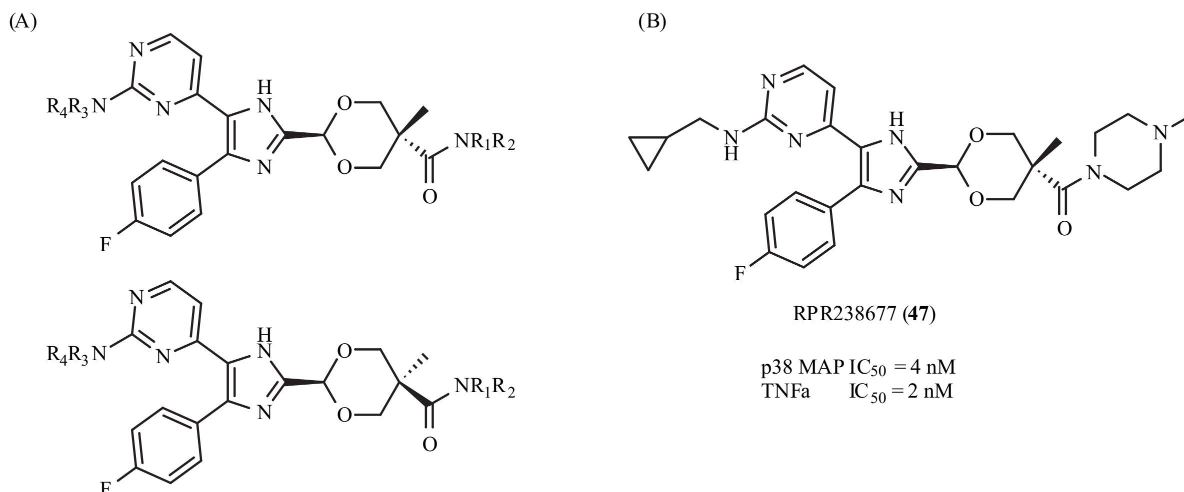

Fig. (28). (A) Target structures for solid-phase combinatorial library for further optimization of RPR200765A **(46).** (B) Structure and activities of RPR238677 (**47**).

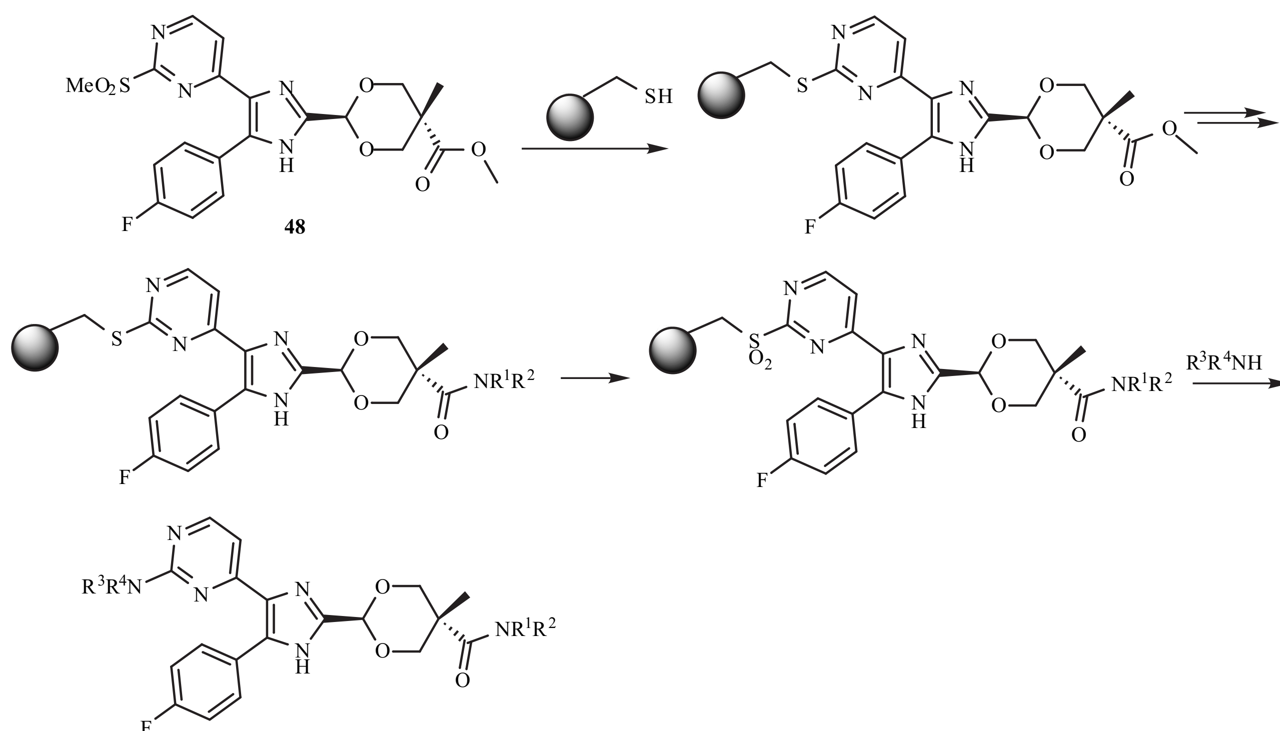

Fig. (29). Solid-phase combinatorial synthesis of imidazole-based p38 MAP kinase inhibitors.

binding site to an adjacent hydrophobic pocket created by the "activation loop" being extended away from the conformation required to catalyze phosphate transfer. Interestingly, it is very common for inhibitors of p38 and Raf to be cross-reactive potentially because both of these kinases can be trapped by inhibitors in an inactive conformation.

As part of a combinatorial chemistry effort at Bayer the novel pyrazolyl urea (**49**) was identified as a reversible p38 inhibitor, (Fig. (**30**)) [74]. To rapidly develop a SAR around initial hit **49**, urea libraries were prepared in a combinatorial fashion by reacting amines with isocyanates in anhydrous DMF (80–95 °C, 18 h). A modular parallel-synthesis work-station, incorporating a Gilson 215 robotic liquid handling and a J-KEM reaction block, allowed the preparation of approximately 1000 analogs, typically as 10x10 reaction matrices. This combinatorial effort in conjunction with the conventional medicinal chemistry allowed a SAR to be rapidly established, (Fig. (**31**)).

The pyrazole unit of **49** could be replaced by an isoxazole, or by a thiophene. The thiadiazole analog is inactive. For the variation of the *tert*-butyl group, very steep structure–activity relationships were observed, with significant potency losses when one carbon atom is either added to or removed from the original tert-butyl group. The phenyl group substitution of t-butyl isoxazolyl ureas is explored, but nothing was found better than the original 2, 3-dichlorophenyl group. Next attention was focused on the 2-position of the pyrazole [75]. Replacement of the methyl by a phenyl as in **53** results in a significant increase in biochemical and cellular potency, with the most potent compounds being the 3-nitro analog and 3-amino analog **54**. These compounds are also very potent in the functional cellular assay (TNF and IL-1 induced IL-6 production in

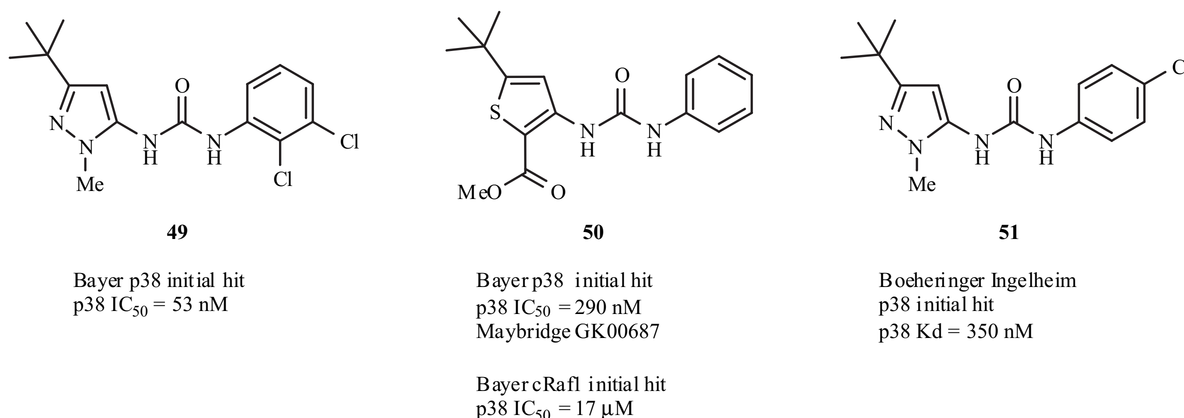

Fig. (30). Hits for p38 and Raf from HTS at Bayer and Boeringer Ingelheim.

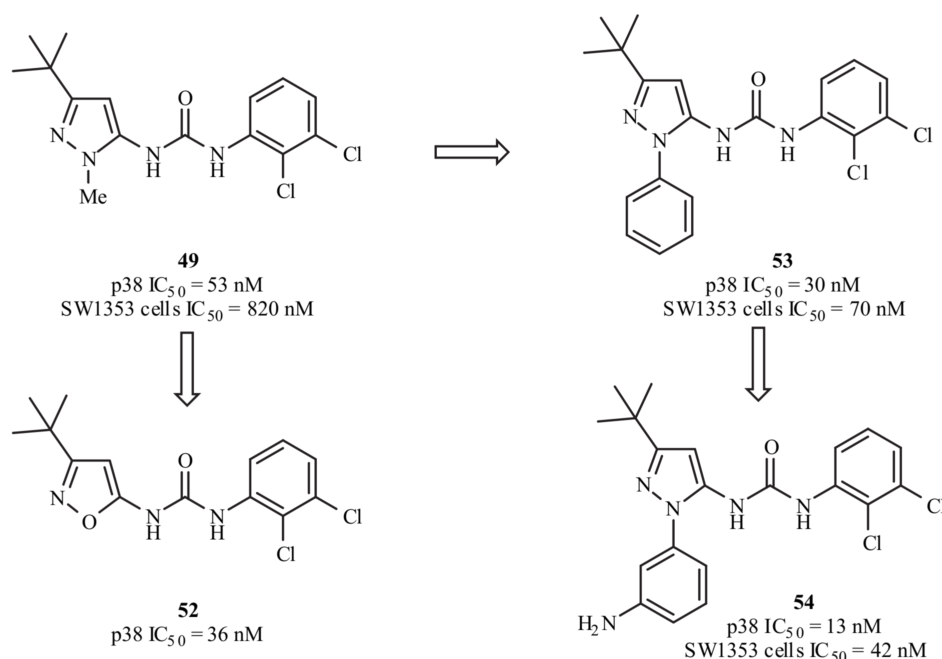

Fig. (31). Optimization of diaryl urea p38 inhibitors.

SW1353 cells). No binding mode for these compounds was reported, but, owing to their striking similarity to BIRB-796 (**42**) (Fig. (**25**)), it will be safe to assume that they belong to the Type II inhibitor class. The selectivity of pyrazole **54** has been assessed against several cytosolic signaling kinases. With the exception of the other p38 isoform, p38 β1 (IC_{50}=52 nM), **54** is only moderately active against JNK-1 (IC_{50}=850 nM) and Abl (IC_{50}=2.7 µM), and inactive against ERK1 (0% inhibition at 5 µM). Testing of **54** against a panel of kinases at MDS Panlabs (Bothell, WA) showed modest potency against HER2 (8.6 µM), $p56^{lck}$ (2.7 µM), and little effect against PKA, PKCα, PKCβ, PKCγ, EGF receptor kinase, and $p59^{fyn}$ (less than 50% inhibition at 10 µM).

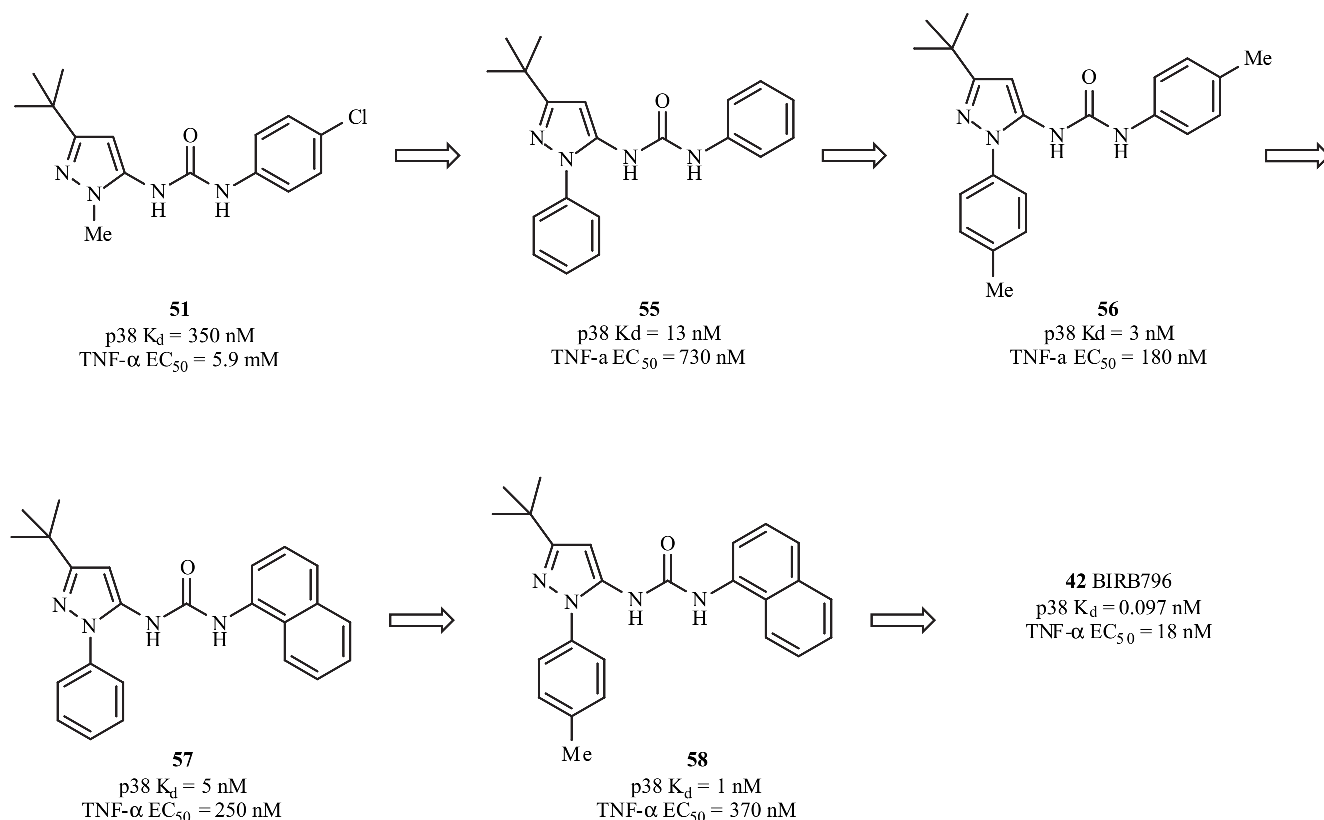

Fig. (32). Optimization of diaryl-urea p38 MAP kinase inhibitor.

The researchers at Boehringer Ingelheim Pharmaceuticals also identified diaryl urea **51** as a modest but novel p38 MAP kinase inhibitor (K_d = 350 nM) from high throughput screening, (Fig. (**30**)) [76]. Despite its modest activity, the hit bore no resemblance to the other known inhibitors at the time and, thus, the binding mode of **51** was determined by x-ray crystallography [9]. The structure revealed that **51** utilizes a new allosteric binding pocket of the kinase, which is spatially distinct from the ATP binding site (see Fig. (**32**) for the schematic binding mode of the optimized compound (**42**) to p38). The iterative optimization cycle closely supported and guided by the x-ray crystallographic structure determinations of several key compounds culminated in the clinical candidate BIRB-796 **(42).** (Fig. (**32**)) summarizes a path from the hit **51** to the final **42**.

BIRB-796 (**42**) demonstrated excellent *in vivo* potency in a mouse model of lipopolysaccharide (LPS)-stimulated TNF- synthesis and other models. When orally dosed to cynomolgous monkeys (10mg/kg), **42** exhibited 1.7 μg/ml, 3.0 hrs, and 80% for Cmax, Tmax, and bioavailability, respectively. BIRB-796 has a very high selectivity profile against a panel of protein kinases; it does not inhibit Erk-1, Fyn, Lck, Syk, PKCα, PKCβ, PKCγ, Zap70, FGFr, Her2, PKA, IKK2β. It inhibits c-Raf-1 (IC_{50} 1.4 μM) and JNK2α2 (IC_{50} 0.1 μM), but still maintains 1000 folds selectivity over p38. On the basis of these and other data, BIRB-796 (**42**) was selected for human clinical trials.

The summary of the binding interaction is shown in (Fig. (**33**)). The carbonyl oxygen and one of the urea NH form hydrogen bonds with Asp168 and Glu71 respectively. The *t*-butyl group is embedded deep into a hydrophobic pocket created by a substantial conformational change to the DFG containing loop. This conformation is very similar to that observed for Gleevec® bound to Abl (type II inhibitor). The tolyl group appendage to the pyrazole ring has a favorable interaction with the alkyl side chain of Glu71 and may contribute to the extensive hydrogen bond network of the urea atoms with the protein by displacing water molecules. The naphthyl ring provides better lipophilic interaction with the specificity pocket and properly aligns the ethoxy morpholine unit for efficient binding into ATP binding pocket. The morpholine oxygen forms a hydrogen bond with Met109 located at the hinge region of the ATP binding site.

B. Diaryl Urea as Raf Kinase Inhibitor

The serine/threonine kinase Raf is an effecter directly downstream of Ras and is involved in the transmission of proliferation signal from the cell surface to the nucleus [77]. The Ras-MAP kinase pathway plays an essential role in cellular proliferation, differentiation, apoptosis, and tumorigenesis. In fact, deregulation of this pathway, either activating mutation of Ras or activating mutation of Raf, is implicated in many cancers. Therefore Raf is an attractive target for a novel cancer therapy [78]. In collaboration with Onyx Pharmaceuticals, researchers at Bayer performed a high throughput screening campaign of 200, 000 compounds against recombinant Raf-1 kinase, leading to an identification of a commercially available thienyl urea **50** as a modest reversible inhibitor (IC_{50} = 17μM), (Fig. (**30**)) [79, 80]. The compound was also identified in their laboratory as a potent p38 inhibitor (p38 IC_{50} = 290nM). As mentioned above, the urea structures were almost unknown as kinase inhibitors around the time of these hit discoveries, and so its novelty and potential for efficient synthesis of analogs made this hit attractive for further investigation. Initial medicinal chemistry efforts optimized the left-hand-side thienyl ring system including its two substituents and the right-hand-side aromatic ring system. In summary, potency of **50** was increased 10-fold by 4-methyl

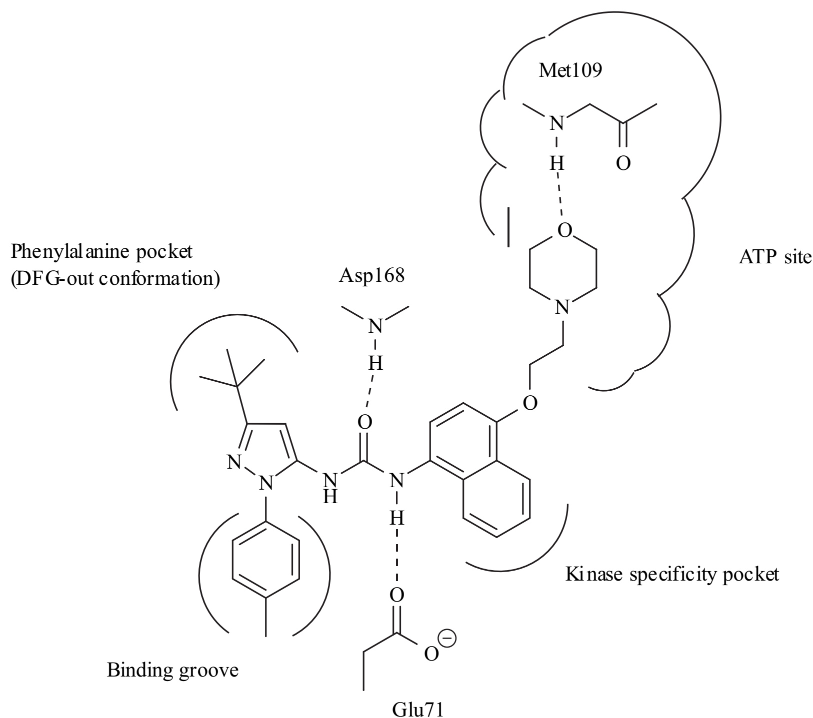

Fig. (33). Summary of binding interaction of BIRB 796 (**42**) with human p38α.

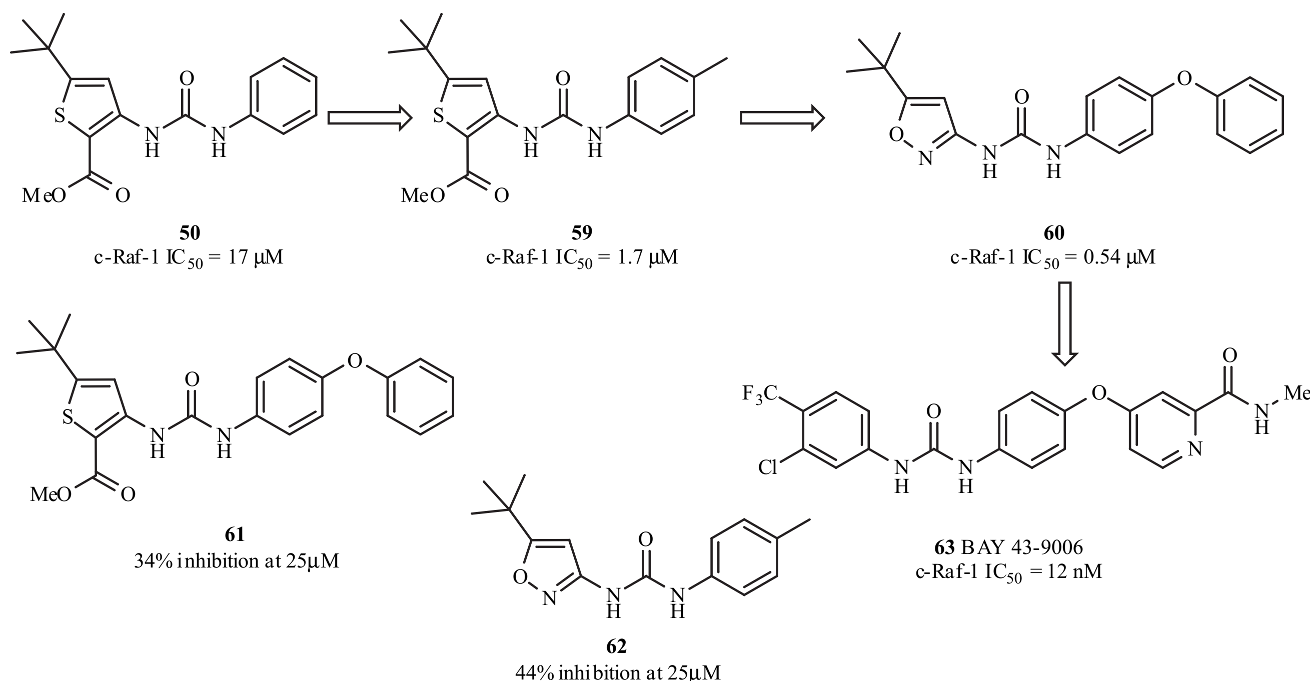

Fig. (34). Optimization of Raf inhibitors.

substitution on the phenyl ring to give analog **59** (IC_{50}=1.7 μM) (Fig. (**34**)). However, despite their efforts of extensive analog synthesis, characterization of SAR, and identification of compounds with potency comparable to **59**, this series could not improved beyond the 1 μM IC_{50} barrier by the sequential medicinal chemical approach. Fortunately, their combinatorial synthesis program, carried out in parallel during the latter stage of this analoging effort, provided a new direction. The combinatorial effort delivered approximately 1000 compounds that were prepared by a modular parallel synthesis workstation as described in the p38 section (see above). One of the library members, **60**, turned out to be the first submicromoler inhibitor (IC_{50}=0.54 μM). This result demonstrates the power of combinatorial approach, for **60** lies outside the SAR established by the sequential analog synthesis, which identified **61** and **62** as relatively inactive inhibitors. Further analoging around **60** rapidly established this compound as the lead for a second generation series of Raf kinase inhibitors with significantly enhanced potential. The following optimization of this new lead to the clinical candidate BAY-43-9006 (**63**) was summarized in (Fig. (**34**)).

CONCLUSION

The past decade has witnessed a significant advancement in our understanding of kinase regulation and in the design of selective small molecule inhibitors. The clinical success of Gleevec® for the treatment of chronic phase chronic myelogenous leukemia (CML) demonstrated that not only can kinase inhibitors be efficacious, but that they could also be well tolerated. Despite this success a significant number of promising kinase inhibitors were abandoned in late preclinical or early preclinical stage due to toxicity and lack of "drug-like" properties. These failures combined with the continued emergence of promising new kinase targets present many future challenges for medicinal chemists. Some of these include: (1) determining which off-target kinases (or other target) effects can be tolerated, (2) determining which combinations of kinases to inhibit for various indications, (3) determining and optimizing the chemical scaffold classes that possess these profiles, and (4) searching for news means of modulating kinase activity through interactions with allosteric sites or regulatory proteins.

ACKNOWLEDGEMENT

We thank Ms Tammy Colbert for her assistance to prepare this manuscript. We also thank the Novartis Research Foundation for a generous funding for Genomics Institute of the Novartis Research Foundation.

ABBREVIATIONS

ACAT	=	Acyl-CoA cholesterol *O*-acyl transferase
ATP	=	Adenosine TriPhosphate
AUC	=	Area under the curve
cAMP	=	Cyclic adenosine monophosphate
CDK	=	Cyclin-Dependent Kinase
CSK	=	carboxyl-termianl Src kinase
Cp	=	Plasma concentration
CYP	=	Cytochrome p450
DARP-32	=	Dopamine and c-AMP-Regulated Phospho-protein-32
EGFR	=	Epidermal Growth Factor Receptor
ERK1	=	Extracellular signal-Regulated Kinase 1 (MAPK3)

FGFR	=	Fibroblast Growth Factor Receptor
IGF1R	=	Insulin-like Growth Factor-1 Receptor
IKK	=	Inhibitor-Kappa-beta Kinase
IL-1β	=	Interleukin-1-β
IL-2	=	Interleukin-2
IL-6	=	Interleukin-6
IRK	=	Insulin Receptor tyrosine Kinase
ITAM	=	Immunoreceptor Tyrosine Activation Motif
JAK3	=	Janus Kinase 3
KDR	=	Kinase insert Domain Receptor (Vascular endothelial growth factor receptor 2)
LCK	=	LymphoCyte-specific protein-tyrosine Kinase
LPS	=	Lipopolysaccharide
MAPK	=	Mitogen-Activated Protein Kinase
MEK	=	MAPK/ERK Kinase
PDGFR	=	Platelete-Derived Growth Factor Receptor
PKA	=	Protein Kinase A (cAMP-dependent protein kinase)
PKB	=	Protein Kinase B (Akt)
PKC	=	Protein Kinase C
SAR	=	Structure Activity Relationship
SH-2	=	Src Homology domain 2
TCR	=	T-Cell antigen Receptor
TNF-α	=	Tumor necrosis factor-α
VLS	=	Virtual ligand screening
ZAP70	=	Zeta-Associated Protein, 70-kD (Zeta-chain-associated protein kinase)

REFERENCES

[1] al-Obeidi, F.A.; Wu, J. J.; Lam, K. S. *Biopolymers*, **1998**, *47*, 197-223.

[2] Chan, A. C.; Desai, D. M.; Weiss, A. *Annu. Rev. Immunol.*, **1994**, *12*, 555-92.

[3] Patarca, R. *Crit. Rev. Oncog.*, **1996**, *7*, 343-432.

[4] Weiss, A.; Littman, D. R. *Cell*, **1994**, *76*, 263-74.

[5] Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science*, **2002**, *298*, 1912-34.

[6] Hanks, S.K.; Quinn, A. M. *Methods Enzymol*., **1991**, *200*, 38-62.

[7] Huse, M.; Kuriyan, J. *Cell*, **2002**, *109*, 275-282.

[8] Nagar, B.; Bornmann, W. G.; Pellicena, P.; Schindler, T.; Veach, D. R.; Miller, W. T.; Clarkson, B.; Kuriyan, J. *Cancer Res*., **2002**, *62*, 4236-43.

[9] Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Grob, P. M.; Hickey, E. R.; Moss, N.; Pav, S.; Regan, J. *Nat. Struct. Biol*., **2002**, *9*, 268-72.

[10] Wan, P. T.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R. *Cell*, **2004**, *116*, 855-67.

[11] Sebolt-Leopold, J. S.; Dudley, D. T.; Herrera, R.; Van Becelaere, K.; Wiland, A.; Gowan, R. C.; Tecle, H.; Barrett, S. D.; Bridges, A.; Przybranowski, S.; Leopold, W. R.; Saltiel, A. R. *Nat. Med.*, **1999**, *5*, 810-6.

[12] Grimsby, J.; Sarabu, R.; Corbett, W. L.; Haynes, N. E.; Bizzarro, F. T.; Coffey, J. W.; Guertin, K. R.; Hilliard, D. W.; Kester, R. F.; Mahaney, P. E.; Marcus, L.; Qi, L.; Spence, C. L.; Tengi, J.; Magnuson, M. A.; Chu, C. A.; Dvorozniak, M. T.; Matschinsky, F. M.; Grippo, J. F. *Science*, **2003**, *301*, 370-3.

[13] Davis, S. D.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.*, **2000**, *351*, 95-105.

[14] Fabbro, D.; Parkinson, D.; Matter, A. *Curr. Opin. Pharm.*, **2002**, *2*, 374-81.

[15] Shah, S. R.; Walsh, T. L.; Williams, C. B.; Soefje, S. A. *J. Oncology Pharmacy Practice*, **2003**, *9*, 151-160.

[16] Traxler, P.; Furet, P. *Pharmacol. Ther.*, **1999**, *82*, 195-206.

[17] Knockaert, M.; Greengard, P.; Meijer, L. *Trends Pharmacol. Sci.*, **2002**. *23*, 417-25.

[18] Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; O'Reilly, T.; Wood, J.; Zimmermann, J. *Med. Res. Rev.*, **2001**, *21*, 499-512.

[19] Bridges, A.J. *Chem. Rev.*, **2001**, *101*, 2541-72.

[20] Fischer, P.M.; Endicott, J.; Meijer, L. *Prog. Cell Cycle Res.*, **2003**, *5*, 235-48.

[21] Garcia-Echeverria, C.; Traxler, P.; Evans, D. B. *Med. Res. Rev.*, **2000**, *20*, 28-57.

[22] Fischer, P.M.; Gianella-Borradori, A. *Exp. Opin. Investig. Drugs*, **2003**, *12*, 955-70.

[23] Malumbres, M.; Barbacid, M. *Nat. Rev. Cancer*, **2001**, *1*, 222-31.

[24] Senderowicz, A. M.; Headlee, D.; Stinson, S. F.; Lush, R. M.; Kalil, N.; Villalba, L.; Hill, K.; Steinberg, S. M.; Figg, W. D.; Tompkins, A.; Arbuck, S. G.; Sausville, E. A. *J. Clin. Oncol.*, **1998**, *16*, 2986-99.

[25] Akinaga, S.; Sugiyama, K.; Akiyama, T. *Anticancer Drug Des.*, **2000**, *15*, 43-52.

[26] Glab, N.; Labidi, B.; Qin, L. X.; Trehin, C.; Bergounioux, C.; Meijer, L. *FEBS Lett.*, **1994**, *353*, 207-11.

[27] Schulze-Gahmen, U.; Brandsen, J.; Jones, H. D.; Morgan, D. O.; Meijer, L.; Vesely, J.; Kim, S. H. *Proteins*, **1995**. *22*, 378-91.

[28] Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P.; Blow, J. J.; Inagaki, N.; Inagaki, M.; Delcros, J. G.; Moulinoux, J. P. *Euro. J. Biochem.*, **1997**, *243*, 527-36.

[29] Meijer, L.; Raymond, E.; *Acc. Chem. Res*., **2003**, *36*, 417-25.

[30] Gray, N. S.; Wodicka, L.; Thunnissen, A. M.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S. H.; Lockhart, D. J.; Schultz, P. *Science*, **1998**, *281*, 533-8.

[31] Davies, T. G.; Bentley, J.; Arris, C. E.; Boyle, F. T.; Curtin, N. J.; Endicott, J. A.; Gibson, A. E.; Golding, B. T.; Griffin, R. J.; Hardcastle, I. R.; Jewsbury, P.; Johnson, L. N.; Mesguiche, V.; Newell, D. R.; Noble, M. E.; Tucker, J. A.; Wang, L.; Whitfield, H. J. *Nat. Struct. Biol.*, **2002**, *9*, 745-9.

[32] Arris, C. E.; Boyle, F. T.; Calvert, A. H.; Curtin, N. J.; Endicott, J. A.; Garman, E. F.; Gibson, A. E.; Golding, B. T.; Grant, S.; Griffin, R. J.; Jewsbury, P.; Johnson, L. N.; Lawrie, A. M.; Newell, D. R.; Noble, M. E.; Sausville, E. A.; Schultz, R.; Yu, W. *J. Med. Chem*., **2000**, *43*, 2797-804.

[33] Dreyer, M. K.; Borcherding, D. R.; Dumont, J. A.; Peet, N. P.; Tsay, J. T.; Wright, P. S.; Bitonti, A. J.; Shen, J.; Kim, S. H. *J. Med. Chem*., **2001**, *44*, 524-30.

[34] Fischer, P.M.; Gianella-Borradori, A. *Exp. Opin. Investig. Drugs*, **2003**, *12*, 955-70.

[35] Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H. Y.; Lu, S.; Qian, L.; Han, W. C.; Shan, W.; Mitt, T.; Cai, Z. W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C. Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.; Marathe, P.; Bursuker, I.; Kellar, K. A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C. R.; Lee, F. Y.; Webster, K. R. *J. Med. Chem.*, **2002**, *45*, 3905-27.

[36] Honma, T.; Hayashi, K.; Aoyama, T.; Hashimoto, N.; Machida, T.; Fukasawa, K.; Iwama, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Iwasawa, Y.; Hayama, T.; Nishimura, S.; Morishima, H. *J. Med. Chem.*, **2001**, *44*, 4615-27.

[37] Honma, T.; Yoshizumi, T.; Hashimoto, N.; Hayashi, K.; Kawanishi, N.; Fukasawa, K.; Takaki, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Hayama, T.; Nishimura, S.; Morishima, H. *J. Med. Chem.*, **2001**, *44*, 4628-40.

[38] Fischer, A.; Sananbenesi, F.; Spiess, J.; Radulovic, J. *Current Drug Targets - Cns & Neurological Disorders*, **2003**, *2*, 375-81.

[39] Dhavan, R.; Tsai, L.H. *Nat. Rev. Mol. Cell Biol.*, **2001**, *2*, 749-59.

[40] Lau, L. F.; Seymour, P. A.; Sanner, M. A.; Schachter, J. B. *J. Mol. Neuroscience*, **2002**, *19*, 267-73.

[41] Svenningsson, P.; Nishi, A.; Fisone, G.; Girault, JA.; Nairn, AC.; Greengard, P. *Annu. Rev. Pharmacol. Toxicol.*, **2004**, *44*, 269-296.

[42] Chergui, K.; Svenningsson, P.; Greengard, P. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*, 2191-6.

[43] Larsen, S. D.; Stachew, C. F.; Clare, P. M.; Cubbage, J. W.; Leach, K. L. *Bioorg. Med. Chem. Lett*., **2003**, *13*, 3491-5.

[44] Changelian, P. S.; Flanagan, M. E.; Ball, D. J.; Kent, C. R.; Magnuson, K. S.; Martin, W. H.; Rizzuti, B. J.; Sawyer, P. S.; Perry, B. D.;Brissette, W. H.; McCurdy, S. P.;Kudlacz, E. M.; Conklyn, M. J.;Elliott, E. A.; Koslov, E. R.; Fisher, M. B.; Strelevitz, T. J.; Yoon, K.; Whipple, D. A.; Sun, J.; Munchhof, M. J.; Doty, J. L.; Casavant, J. M.; Blumenkopf, T. A.; Hines, M.; Brown, M. F.; Lillie, B. M.; Subramanyam, C.; Shang-Poa, C.; Milici, A. J.; Beckius, G. E.; Moyer, J. D.; Su, C.; Woodworth, T. G.; Gaweco, A. S.; Beals, C. R.; Littman, B. H.; Fisher, D. A.; Smith, J. F.; Zagouras, P.; Magna, H. A.; Saltarelli, M. J.; Johnson, K. S.; Nelms, L. F.; Des Etages, S. G.; Hayes, L. S.; Kawabata, T. T.; Finco-Kent, D.; Baker, D. L.; Larson, M.; Si, M. S.; Paniagua, R.; Higgins, J.; Holm, B.; Reitz, B.; Zhou, Y. J.;Morris, R. E.;O'Shea, J. J.; Borie, D. C. *Science*, **2003**, *302*, 875-8.

[45] Adams, C.; Aldous, D. J.; Amendola, S.; Bamborough, P.; Bright, C.; Crowe, S.; Eastwood, P.; Fenton, G.; Foster, M.; Harrison, T. K.; King, S.; Lai, J.; Lawrence, C.; Letallec, J. P.; McCarthy, C.; Moorcroft, N.; Page, K.; Rao, S.; Redford, J.; Sadiq, S.; Smith, K.; Souness, J. E.; Thurairatnam, S.; Vine, M.; Wyman, B. *Bioorg. Med. Chem. Lett.*, **2003**. *13*, 3105-10.

[46] Molina, T. J.; Kishihara, K.; Siderovski, D. P.; van Ewijk, W.; Narendran, A.; Timms, E.; Wakeham, A.; Paige, C. J.; Hartmann, K. U.; Veillette, A.; Davidson, D.; Mak, T.W. *Nature*, **1992**, *357*, 161-4.

[47] Levin, S. D.; Anderson, S. J.; Forbush, K. A.; Perlmutter, R. M. *Embo J*., **1993**, *12*, 1671-80.

[48] Hanke, J. H.; Gardner, J. P.; Dow, R. L.; Changelian, P. S.; Brissette, W. H.; Weringer, E. J.; Pollok, B. A.; Connelly, P. A. *J. Biol. Chem.*, **1996**, *271*, 695-701.

[49] van Oers, N. S.; Lowin-Kropf, B.; Finlay, D.; Connolly, K.; Weiss, A. *Immunity*, **1996**, *5*, 429-36.

[50] Padmanabha, R.; Shu, Y. Z.; Cook, L. S.; Veitch, J. A.; Donovan, M.; Lowe, S.; Huang, S.; Pirnik, D.; Manly, S. P. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 569-74.

[51] Bullington, J. L.; Cameron, J. C.; Davis, J. E.; Dodd, J. H.; Harris, C. A.; Henry, J. R.; Pellegrino-Gensey, J. L.; Rupert, K. C.; Siekierka, J. J. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2489-94.

[52] Kraker, A. J.; Hartl, B. G.; Amar, A. M.; Barvian, M. R.; Showalter, H. D.; Moore, C. W. *Biochem. Pharmacol.*, **2000**, *60*, 885-98.

[53] Arnold, L. D.; Calderwood, D. J.; Dixon, R. W.; Johnston, D. N.; Kamens, J. S.; Munschauer, R.; Rafferty, P.; Ratnofsky, S. E. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2167-70.

[54] Burchat, A. F.; Calderwood, D. J.; Hirst, G. C.; Holman, N. J.; Johnston, D. N.; Munschauer, R.; Rafferty, P.; Tometzki, G. B. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2171-4.

[55] Chen, P.; Norris, D.; Iwanowicz, E. J.; Spergel, S. H.; Lin, J.; Gu, H. H.; Shen, Z.; Wityak, J.; Lin, T. A.; Pang, S.; De Fex, H. F.; Pitt, S.; Shen, D. R.; Doweyko, A. M.; Bassolino, D. A.; Roberge, J. Y.; Poss, M. A.; Chen, B. C.; Schieven, G. L.; Barrish, J. C. *Bioorg. Med. Chem. Lett.*, **2002**. *12*, 1361-4.

[56] Das, J.; Lin, J.; Moquin, R. V.; Shen, Z.; Spergel, S. H.; Wityak, J.; Doweyko, A. M.; DeFex, H. F.; Fang, Q.; Pang, S.; Pitt, S.; Shen, D. R.; Schieven, G. L.; Barrish, J. C. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 2145-9.

[57] Chen, P.; Iwanowicz, E. J.; Norris, D.; Gu, H. H.; Lin, J.; Moquin, R. V.; Das, J.; Wityak, J.; Spergel, S. H.; de Fex, H.; Pang, S.; Pitt, S.; Shen, D. R.; Schieven, G. L.; Barrish, J. C. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 3153-6.

[58] Wityak, J.; Das, J.; Moquin, R. V.; Shen, Z.; Lin, J.; Chen, P.; Doweyko, A. M.; Pitt, S.; Pang, S.; Shen, D. R.; Fang, Q.; de Fex, H. F.; Schieven, G. L.; Kanner, S. B.; Barrish, J. C. *Bioorg. Med. Chem. Lett.*, **2003**. *13*, 4007-10.

[59] Das, J.; Moquin, R. V.; Lin, J.; Liu, C.; Doweyko, A. M.; DeFex, H. F.; Fang, Q.; Pang, S.; Pitt, S.; Shen, D. R.; Schieven, G. L.; Barrish, J. C.; Wityak, J. *Bioorg. Med. Chem. Lett.*, **2003**. *13*, 2587-90.

[60] Kumar, S.; Boehm, J.; Lee, J.C. *Nat. Rev. Drug Disc.*, **2003**, *2*, 717-26.

[61] Lee, J. C.; Badger, A. M.; Griswold, D. E.; Dunnington, D.; Truneh, A.; Votta, B.; White, J. R.; Young, P. R.; Bender, P. E. *Ann. N. Y. Acad. Sci.*, **1993**, *696*, 149-70.

[62] Muller-Ladner, U. *Curr. Opin. Rheumatol.*, **1996**, *8*, 210-20.

[63] Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallgher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Keys, J. R.; Landvatter, S. W.; Stricker, J. E.; McLaughliln, M. M.; Siemens, I. R.; Fisher, S. M.; Livo, G. P.; White, J. R.; Adams, J. L.; Young, P. R. *Nature*, **1994**, *372*, 739-46.

[64] Han, J.; Lee, J. D.; Bibbs, L.; Ulevitch, R. J. *Science*, **1994**, *265*, 808-11.

[65] Ward, K. W.; Proksch, J. W.; Salyers, K. L.; Azzarano, L. M.; Morgan, J. A.; Roethke, T. J.; McSurdy-Freed, J. E.; Levy, M. A.; Smith, B. R. *Xenobiotica*, **2002**, *32*, 221-33.

[66] Fijen, J. W.; Zijlstra, J. G.; De Boer, P.; Spanjersberg, R.; Tervaert, J. W.; Van Der Werf, T. S.; Ligtenberg, J. J.; Tulleken, J. E. *Clin. Exp. Immunol.*, **2001**, *124*, 16-20.

[67] Goldstein, D.M.; *J. Inflamm. Res.*, **2002**, *51*, S114.

[68] Wood, C. C.; Yong, C. L.; Madwed, J. B, Gupta, A. *58th Ann. Meet. Am. Acad. Allergy, Asthma Immunol.,* **2002**, *109*, S321.

[69] Gupta, A. *58th Ann. Meet. Am. Acad. Allergy, Asthma Immunol.*, **2002**, *109*, S67.

[70] Polmar, S.H.; *58th Ann. Meet. Am. Acad. Allergy, Asthma Immunol.,* **2002**, *109*, S66.

[71] Weisman, M. *Ann. Euro. Congress Rheumatol.*, **2002**, A10018.

[72] McLay, L. M.; Halley, F.; Souness, J. E.; McKenna, J.; Benning, V.; Birrell, M.; Burton, B.; Belvisi, M.; Collis, A.; Constan, A.; Foster, M.; Hele, D.; Jayyosi, Z.; Kelley, M.; Maslen, C.; Miller, G.; Ouldelhkim, M. C.; Page, K.; Phipps, S.; Pollock, K.; Porter, B.; Ratcliffe, A. J.; Redford, E. J.; Webber, S.; Slater, B.; Thybaud, V.; Wilsher, N. *Bioorg. Med. Chem*., **2001**, *9*, 537-54.

[73] McKenna, J. M.; Halley, F.; Souness, J. E.; McLay, I. M.; Pickett, S. D.; Collis, A. J.; Page, K.; Ahmed, I. *J. Med. Chem.*, **2002**, *45*, 2173-84.

[74] Dumas, J.; Sibley, R.; Riedl, B.; Monahan, M. K.; Lee, W.; Lowinger, T. B.; Kingery-Wood, J.; Scott, W. J.; Smith, R. A.; Bobko, M.; Schoenleber, R.; Ranges, G. E.; Housley, T. J.; Bhargava, A.; Wilhelm, S. M.; Shrikhande, A. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2047-50.

[75] Dumas, J.; Hatoum-Mokdad, H.; Sibley, R.; Riedl, B.; Scott, W. J.; Monahan, M. K.; Lowinger, T. B.; Brennan, C.; Natero, R.; Turner, T.; Johnson, J. S.; Schoenleber, R.; Bhargava, A.; Wilhelm, S. M.; Housley, T. J.; Ranges, G. E.; Shrikhande, A. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 2051-4.

[76] Regan, J.; Breitfelder, S.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Hickey, E.; Klaus, B.; Madwed, J.; Moriak, M.; Moss, N.; Pargellis, C.; Pav, S.; Proto, A.; Swinamer, A.; Tong, L.; Torcellini, C. *J. Med. Chem.*, **2002**, *45*, 2994-3008.

[77] Lowy, D.R.; Willumsen, B.M. *Annu. Rev. Biochem*., **1993**, *62*, 851-91.

[78] Naumann, U.; Eisenmann-Tappe, I.; Rapp, U.R. *Recent Results Cancer Res.*, **1997**, *143*, 237-44.

[79] Smith, R. A.; Barbosa, J.; Blum, C. L.; Bobko, M. A.; Caringal, Y. V.; Dally, R.; Johnson, J. S.; Katz, M. E.; Kennure, N.; Kingery-Wood, J.; Lee, W.; Lowinger, T. B.; Lyons, J.; Marsh, V.; Rogers, D. H.; Swartz, S.; Walling, T.; Wild, H. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2775-8.

[80] Lowinger, T. B.; Riedl, B.; Dumas, J.; Smith, R. A. *Curr. Pharm. Des.*, **2002**, *8*, 2269-78.

Received: 19 March, 2004 **Accepted: 07 June, 2004**