REVIEW

Kinase inhibitors for the treatment of inflammatory and autoimmune disorders

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Abstract Drugs targeting inhibition of kinases for the treatment of inflammation and autoimmune disorders have become a major focus in the pharmaceutical and biotech industry. Multiple kinases from different pathways have been the targets of interest in this endeavor. This review describes some of the recent developments in the search for inhibitors of IKK2, Syk, Lck, and JAK3 kinases. It is anticipated that some of these compounds or newer inhibitors of these kinases will be approved for the treatment of rheumatoid arthritis, psoriasis, organ transplantation, and other autoimmune diseases.

Introduction

Discovery of novel drugs targeting kinases, an important class of intracellular enzymes that play a critical role in signal transduction pathways controlling a variety of cellular functions, has become the focus of a large number of drug discovery programs in the pharmaceutical and biotech industry. The role of a kinase in signal transduction is to catalyze the transfer of the terminal phosphate group of ATP to an appropriate substrate leading to the activation of the substrate for its role in the next step of the signaling cascade. The substrate is often another kinase or a transcription factor. A large majority of kinase inhibitors are designed to inhibit the enzyme by binding at or near the ATP-binding site. Therefore, an inhibitor of one kinase is often found to inhibit other structurally related or unrelated kinases. This inherent promiscuity of kinase inhibitors calls for extensive profiling of the inhibitors either for driving structure-activity relationship (SAR) during lead optimization or for opportunistic discoveries [1, 2].

Currently eight small molecule kinase inhibitor drugs and a handful of protein/antibody therapeutics targeting kinases have been approved for human use. A large number of kinase inhibitor discovery programs have been focused on drugs for the treatment of inflammation and autoimmune disorders; however, the approved drugs to date have been useful for the treatment of a variety of cancers in humans. One of the reasons cited for this lack of success to date for kinase inhibitor drugs for the treatment of patients with inflammation and autoimmune disorders has been the high hurdle for safety required for the chronic treatment of patients whose life expectancy is usually significantly longer than that of cancer patients.

A large number of kinases from different signal transduction pathways have been the targets of interest for the treatment of inflammation and autoimmune disorders. One class of such kinases have been the mitogen-activated protein kinases (MAP kinases), which has been summarized in a recent review [3], and hence will not be covered in this chapter. This review will cover the recent publications, primarily from 2006–2007, describing inhibitors of IKK2 (IκB kinase 2), Syk (spleen tyrosine kinase), Lck (lymphocyte-specific kinase), and JAK3 (Janus kinase 3). Inhibitors of kinases such as BTK and Fyn are not covered in this review. Some of the publications cited in this review refer to the inhibitors reported earlier for that kinase. A large number of patents on kinase inhibitors describe,

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sometimes with very little, if any, information on the biological profile of compounds. This chapter will not cover such disclosures. Additionally, a majority of kinase inhibitors disclosed as having inhibitory activity for one kinase are found to be potent inhibitors of other kinases. This review will focus on the kinase inhibitors that are profiled for a particular kinase with potential application in inflammation. The inhibitors described for certain kinases, such as Src family kinases, that inhibit other kinases relevant to inflammation, but are not profiled for anti-inflammatory activity, are not included in this review.

IKK2 inhibitors

Nuclear factor (NF)-κB is an important transcription factor that regulates multiple cell functions. This transcription factor exists in the cytoplasm in an inactive form because of its binding to the inhibitory protein, IκB. Upon activation of the cell following the binding of cytokines and growth factors or by UV or stress, a signal transduction cascade unravels that leads to the activation of the serine-threonine kinases, IKK1 and IKK2. IKK2 phosphorylates the Ser32 and Ser36 residues of IκB bound to NF-κB. The phosphorylated complex is ubiquitinated by E3RS ligase and degraded by proteasome to generate the active NF-κB. The transcription factor then translocates to the nucleus and induces the transcription of proinflammatory cytokines and matrix metalloproteases [4]. Inhibition of IKK2 has been pursued as a potential therapy to treat disorders related to inflammation and autoimmunity. Based on the critical role of NF-κB in the immune system and on the data from knockout mice, it has been postulated that chronic inhibition of this transcription factor could lead to opportunistic infections and hepatic toxicity. However, studies in transgenic mice and of some of the inhibitors in animals have indicated that inhibition of NF-κB function is unlikely to cause systemic infection and apoptosis of hepatic tissue in animals [5].

The IKK2 inhibitors discussed herein are shown in Fig. 1. BMS-345541 is reported to be a selective and ATP-noncompetitive inhibitor of IKK2 with $IC_{50}=300$ nM [6]. The compound was not a potent inhibitor of IKK1 ($IC_{50}=4$ μM). In lipopolysaccharide (LPS)-stimulated THP-1 cells, the expression of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α) was inhibited with $IC_{50}=1-5$ μM. At a dose of 30 mg/kg administered once daily, BMS-345541 maximally reduced disease severity in a murine model of dextran sulfate sodium-induced colitis [7]. The compound dosed at 100 mg/kg (po, qd) in this model showed a similar benefit. Structural modification of BMS-345541 has resulted in

compounds **1**–**3**, which are significantly more potent inhibitors of IKK2 with $IC_{50}=10-60$ nM [8]. In LPS-stimulated THP-1 cells, compound **1** inhibited TNF-α production with $IC_{50}=0.34$ μM, while BMS-345541 was less potent in this test with $IC_{50}=4$ μM. Oral administration of compound **1** to mice inhibited the LPS-induced TNF-α levels in the serum with $ED_{50}=10$ mg/kg. A structurally related, imidazo-thieno-pyrazine derivative, **4**, has been reported to inhibit IKK2 with $IC_{50}=13$ nM and IKK1 with $IC_{50}=390$ nM [9]. A 10 mg/kg oral administration of **4** to mice, 1 h prior to LPS challenge, inhibited TNF-α levels by 50%. However, administration of **4**, 4 h prior to LPS challenge, did not inhibit TNF-α levels, indicating that the compound has a short half-life.

A series of 2-anilino-4-arylpyrimidines such as compound **5** have been reported to be potent IKK2 inhibitors with $IC_{50}=11$ nM for compound **5** [10]. The authors have not disclosed cellular and in vivo activity profiles of the compounds and have attempted to explain the SAR using a homology model of IKK2 and using quantitative structure-activity relationship (QSAR) models.

In a series of publications, Murata and coworkers have disclosed optimization of substituted pyridines to identify compound **6** with IKK2 $IC_{50}=8.5$ nM [11–13]. Compound **6** was a poor inhibitor of IKK1 with $IC_{50}=250$ nM. Compound **6** inhibited LPS-induced TNF-α production in human PBMCs (peripheral blood mononuclear cells) with $IC_{50}=50$ nM. Oral administration of 0.3–3 mg/kg of compound **6** inhibited the arachidonic acid-induced ear edema in mice in a dose-dependent manner. The anti-inflammatory activity of **6** at 1 mg/kg oral dose in this model was superior to that of dexamethasone at 0.3 mg/kg oral dose. The oral bioavailability of **6** in rats was 60% with low clearance (CL=0.33 l/h per kg). Compound **7** has been reported to be a potent, ATP-competitive, and moderately selective inhibitor of IKK2 with $K_i=2$ nM [14]. The compound inhibited the cytokines and other inflammatory mediators in a variety of cells upon induction ($IC_{50}=47$ nM for LPS-induced TNF-α production in PBMCs). Compound **7** had good bioavailability in rats and mice and showed beneficial effects in animal models of allergy, lung inflammation, edema, and delayed-type hypersensitivity (DTH).

Structural modification of SC-415, a known weak but selective IKK2 inhibitor [15], has yielded compound **8** and analogs with modest IKK2 inhibitory potency [16]. Compound **8**, with $IC_{50}=333$ nM for inhibition of IKK2, inhibited IL-8 production in IL-1β-stimulated synovial fibroblasts derived from rheumatoid arthritis patients with $IC_{50}=832$ nM.

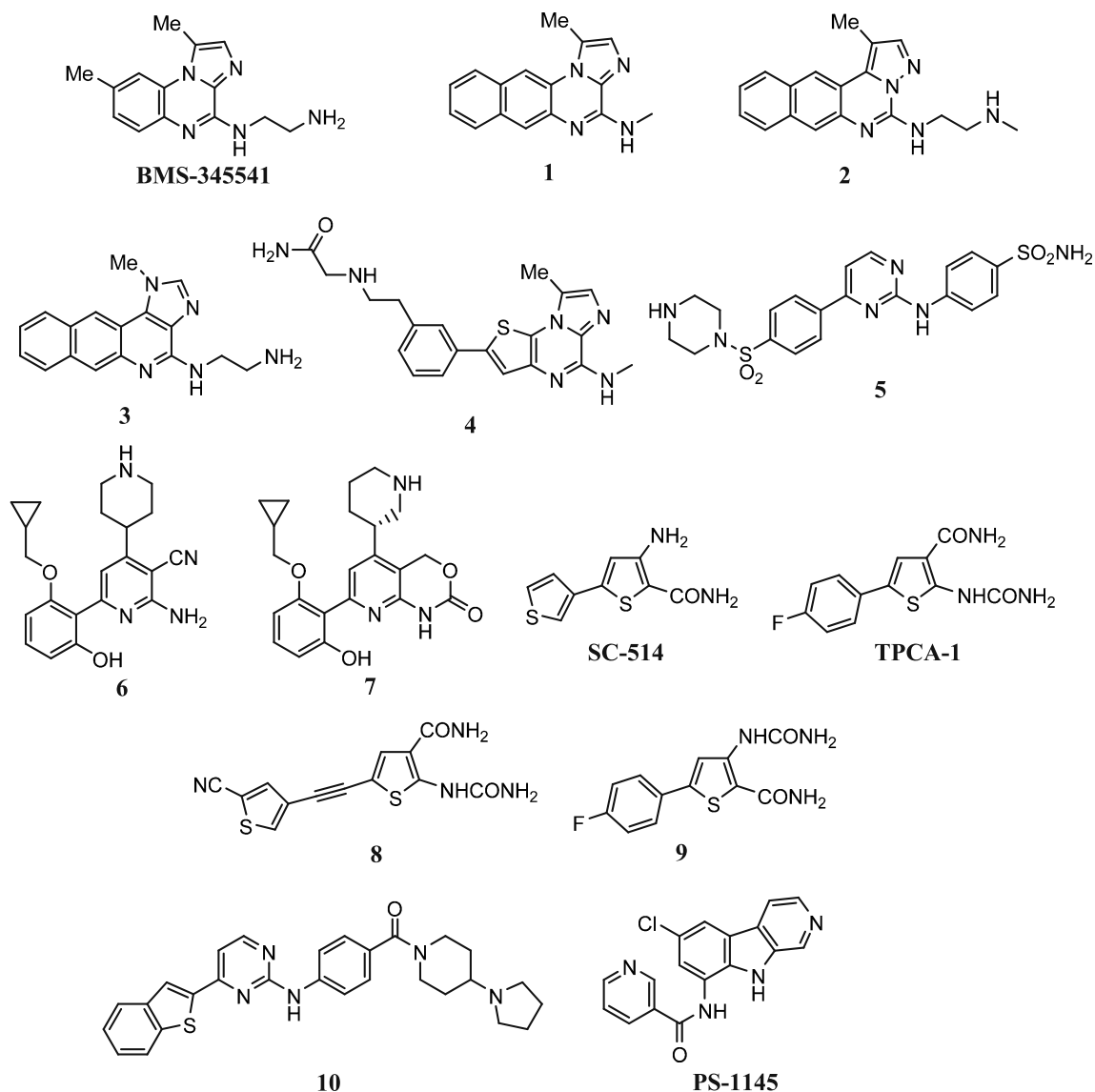A structurally related compound TPCA-1 has been reported to be an ATP-competitive and selective inhibitor

Fig. 1 IKK2 inhibitors

of IKK2 with $IC_{50}=18$ nM [17]. The production of cytokines such as TNF-α, IL-6, and IL-8 induced by LPS in human PBMCs was inhibited by TPCA-1 with $IC_{50}=170$-320 nM. A 20 mg/kg oral dose of TPCA-1 administered twice daily to mice significantly reduced the clinical score and disease severity in a collagen-induced arthritis (CIA) model. Compound **9**, an isomer of TPCA-1, has been reported to be a potent inhibitor of IKK2 with $IC_{50}=63$ nM and 100-fold selective over IKK1 [18]. In PBMCs, the LPS-induced TNF-α production was inhibited by **9** with $IC_{50}=400$ nM. The compound showed low in vitro metabolic clearance in rat hepatocytes, low in vitro plasma protein binding, and good oral bioavailability (%F=78 in rats).

An anilinopyrimidine derivative, **10**, has been reported to be a potent IKK2 inhibitor with $IC_{50}=40$ nM [19]. In

human vascular endothelial cells (Huvec), **10** inhibited the TNF-α-induced expression of the adhesion molecules ICAM-1 and VCAM-1 with $IC_{50}=300$ nM. Administration of 30 mg/kg oral dose of **10** inhibited TNF-α release by 75% upon LPS challenge in rats. Compound **10** exhibited anti-inflammatory activity in a thioglycollate-induced peritonitis model in mice. At a dose of 10 mg/kg s.c. (subcutaneous), **10** inhibited neutrophil extravasation by 50% in this model.

SPC-839, whose structure is undisclosed, has been reported to be a potent and selective IKK2 inhibitor ($IC_{50}=20$ nM) with a significant oral anti-inflammatory activity in an adjuvant-induced arthritis model in rats [20]. The compound has been licensed to Serono and the publications from this company disclose this compound as AS602868 which is an anilinopyrimidine derivative [21].

PS-1145 has been reported to be a potent IKK2 inhibitor with $IC_{50}=100$ nM [22]. The compound inhibited the phosphorylation of the endogenous IKK complex in cell lysates from TNF-α-induced HeLa cells with $IC_{50}=150$ nM. PS-1145, at an oral dose of 50 mg/kg, inhibited LPS-induced TNF-α levels in mice by 60%.

Syk inhibitors

Spleen tyrosine kinase (Syk) is a cytosolic protein tyrosine kinase that plays a crucial role in the IgE (FcϵRI) and IgG (Fcγ) receptor-mediated signaling in mast cells, basophils, and macrophages leading to degranulation and cytokine release that contribute to proinflammatory and allergic responses. In addition, activation of Syk is involved in B-cell receptor signaling as well as Fc receptor-mediated antigen presentation. A variety of experimental evidence points to the potential use of Syk inhibitors in the treatment of various autoimmune disorders [23].

Figure 2 shows the structure of Syk inhibitors discussed below. The oxindoles **11a** and **11b** have been reported to inhibit Syk with $IC_{50}=20$ and 145 nM, respectively [24]. The degranulation of rat basophilic cells (RBL-2H3), induced by IgE/FcϵRI, was inhibited by **11a** and **11b** with $IC_{50}=110$ and 100 nM, respectively.

Compound **12** and analogs have been reported to be potent inhibitors of Syk with no additional data in cells or animals ($IC_{50}=8$ nM for **12**) [25].

BAY 61-3606 has been reported to be an ATP-competitive and selective inhibitor of Syk with $IC_{50}=10$ nM [26]. The degranulation of the RBL-2H3 cell line was inhibited with $IC_{50}=46$ nM. In an ovalbumin-induced

airway inflammation model in the rat, the efficacy of BAY 61-3606, at a dose of 30 mg/kg, b.i.d., in suppressing the accumulation of eosinophils in BAL fluid was similar to that of 0.3 mg/kg po, b.i.d., of dexamethasone. The less than adequate pharmacokinetic profile of BAY 61-3606 (low AUC and fast elimination half-life) contributed to the need for the high dose in rats for efficacy of this potent inhibitor of Syk.

Compound **13** has been reported to be a potent and selective Syk inhibitor with $IC_{50}=41$ nM [27]. The compound inhibited the degranulation of RBL-2H3 cells (as measured by release of serotonin) with $IC_{50}=460$ nM and inhibited the IgE-induced passive cutaneous anaphylaxis reaction in mice with $ED_{50}=13.2$ mg/kg s.c.

R112 and R406, two structurally related analogs, have been reported to be potent, selective, and ATP-competitive inhibitors of Syk. R112 inhibited Syk enzyme activity with $K_i=96$ nM and inhibited anti-IgE-mediated histamine release from primary human basophils with $EC_{50}=280$ nM [28]. In a phase II study in normal volunteers with seasonal allergic rhinitis, intranasally delivered R112 significantly reduced clinical symptoms such as stuffy, itchy, and runny nose, sneezes, cough, and headache [29]. R406 inhibited Syk with $K_i=30$ nM and inhibited anti-IgE-induced degranulation and production and release of leukotrienes, cytokines, and chemokines from cultured human mast cells with $EC_{50}=40$-160 nM [30]. In a CIA model in rats, a 30 mg/kg oral b.i.d dose of R406, or a water-soluble prodrug, R788, completely suppressed symptoms of inflammation and regressed arthritic score including joint destruction [31]. In healthy human volunteers, orally administered R406 was well tolerated, exhibited desirable pharmacokinetic properties, and inhibited baso-
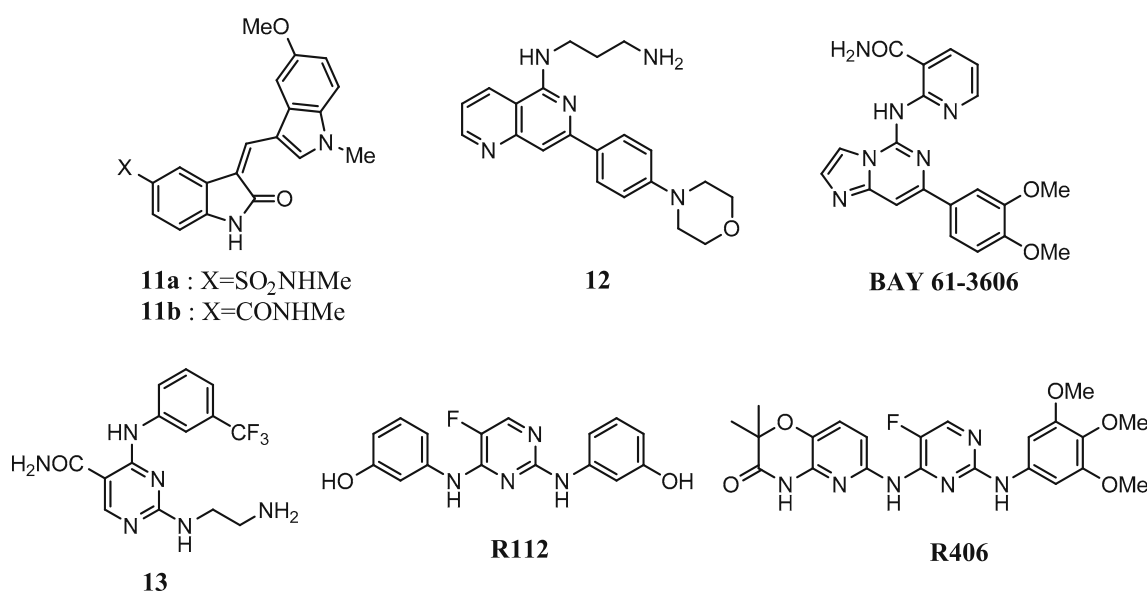

11a : X=SO$_2$NHMe
11b : X=CONHMe

12

BAY 61-3606

13

R112

R406

Fig. 2 Syk inhibitors

phil activation and degranulation induced ex vivo by IgE in a dose-dependent manner.

Lck inhibitors

The lymphocyte-specific kinase (Lck), belonging to the Src family of tyrosine kinases, is expressed in T cells and natural killer (NK) cells and is responsible for the activation of and signaling through the T-cell receptor. Activation of this cascade results in the upregulation of inflammatory cytokines such as IL-2 and interferon (IFN)-γ, and ultimately in the activation and proliferation of T lymphocytes to generate an immune response. Therefore, inhibition of Lck is likely to elicit an immunosuppressive effect that could be useful in the treatment of T-cell-mediated diseases like rheumatoid arthritis, inflammatory bowel disease, psoriasis, and organ graft rejection [32]. A large number of compounds are reported to be potent inhibitors of Lck (see reference 5 in reference [44] in this review). This review will focus on the Lck inhibitors reported primarily in the years 2006–2007 and these publications refer to the earlier reports on Lck inhibitors. There are a number of disclosures of Src or Src family inhibitors as anticancer agents that have or are likely to have Lck inhibitory activity. Most of these compounds are not covered in this review.

Figure 3 summarizes the structure of Lck inhibitors discussed here. An anilinopyrimidine, **14**, has been reported to inhibit Lck with $IC_{50}=19$ nM with a selectivity of 3- to 30-fold against Btk, Lyn, Syk, and Txk and is proposed to bind in the ATP site of Lck [33]. The pharmacokinetic profile of **14** was determined to be modest.

A series of 2,3-diaryl-furopyrimidines have been reported to be modestly selective Lck inhibitors [34]. Compound **15** inhibited Lck with $IC_{50}=98$ nM and inhibited anti-CD3/CD-28-induced secretion of IL-2 in T cells isolated from human peripheral blood lymphocytes with $IC_{50}=430$ nM. The X-ray structure of a close analog of **15** in Lck indicated that the compound binds in the ATP site and that the C-H at the 2-position donates an H-bond to the carbonyl of Glu317. Compound **16**, which is closely related to **15**, is a modestly selective inhibitor of Lck with $IC_{50}=22$ nM. The binding mode and H-bonding pattern of this class of furopyridines in Lck is shown to be similar to that of the furopyrimidines [35].

Compound **17** is reported to be a modestly potent inhibitor of Lck ($IC_{50}=210$ nM) with significant selectivity against the other members of the Src family of kinases [36]. The compound, which had modest oral bioavailability in rats, inhibited anti-CD3 antibody-induced IL-2 production in mice with $ED_{50}=5$ mg/kg po. A structurally related compound, A-770041, is an inhibitor of Lck ($IC_{50}=147$ nM) with a significant selectivity against other members of the Src family of kinases [37]. The anti-CD3 antibody-stimulated IL-2 production in human whole blood was inhibited by this compound with $IC_{50}=80$ nM. A-770041 exhibited a desirable oral pharmacokinetic profile in rats and oral efficacy against heart transplant rejection in a rat model at 10 mg/kg b.i.d. dosing.

Compound **18** (racemic) is reported to be a potent inhibitor of Src ($IC_{50}=20$ nM) and Lck ($IC_{50}=8$ nM) with protective effects (reduction of infarct size) in a rat model of middle cerebral artery occlusion (MCAO) [38]. A molecular modeling-guided design of Src inhibitors has led to the identification of **19** (Src $IC_{50}=28$ nM and Lck $IC_{50}=29$ nM) with efficacy in tumor xenograft models in mice upon intraperitoneal (i.p.) administration [39].

A series of benzimidazole-substituted anilinopyrimidines have been reported to be potent inhibitors of Lck. Compound **20** inhibited Lck with $IC_{50}=3$ nM and inhibited phorbol myristate acetate (PMA)-induced IL-2 production in Jurkat T cells with $IC_{50}=54$ nM [40]. However, the series of compounds seemed to lack specificity against other Src family kinases and lacked desirable pharmacokinetic properties. The pyrimidopyrazine derivative, **21**, is reported to be a potent Lck inhibitor with $IC_{50}=2$ nM [41]. The cellular activity (IL-2 inhibition in T cells), selectivity against other Src family of kinases, and pharmacokinetic properties of **21** were less than optimal.

The anilinopyrimidine urea, **22**, inhibited Lck with $IC_{50}=87$ nM and inhibited the hind paw swelling by 63% upon oral administration twice a day at 25 mg/kg in an adjuvant-induced arthritis model in rats [42].

Compound **23**, a close structural analog of dasatinib (BMS-354825), a marketed kinase inhibitor drug for the treatment of chronic myelogenous leukemia (CML), is a potent, selective, and ATP-competitive inhibitor of Lck and other Src family kinases ($IC_{50}=1$ nM for hLck) [43]. In an ex vivo anti-CD3/CD-28-induced IL-2 production model in mice, orally administered **23** reduced serum IL-2 levels in a dose-dependent manner with $ED_{50}=5$ mg/kg. Compound **23**, which has a desirable pharmacokinetic profile in rats (% F=65), was efficacious in reducing paw swelling upon oral dosing at 3 mg/kg b.i.d. in a rat adjuvant arthritis model of established disease.

The 2-amino-6-aryl-quinazoline derivative, **24**, is a potent Lck inhibitor ($IC_{50}=0.5$ nM) that is not selective against other members of Src family kinases, p38, and VEGFR2 [44]. In a human whole blood assay, **24** inhibited the anti-CD3/CD28 antibody-induced IL-2 production with $IC_{50}=113$ nM. Compound **24** had a desirable pharmacokinetic profile in rats (%F=55) and was orally efficacious in reducing serum levels of IL-2 in BALB/c mice with $ED_{50}=22$ mg/kg.
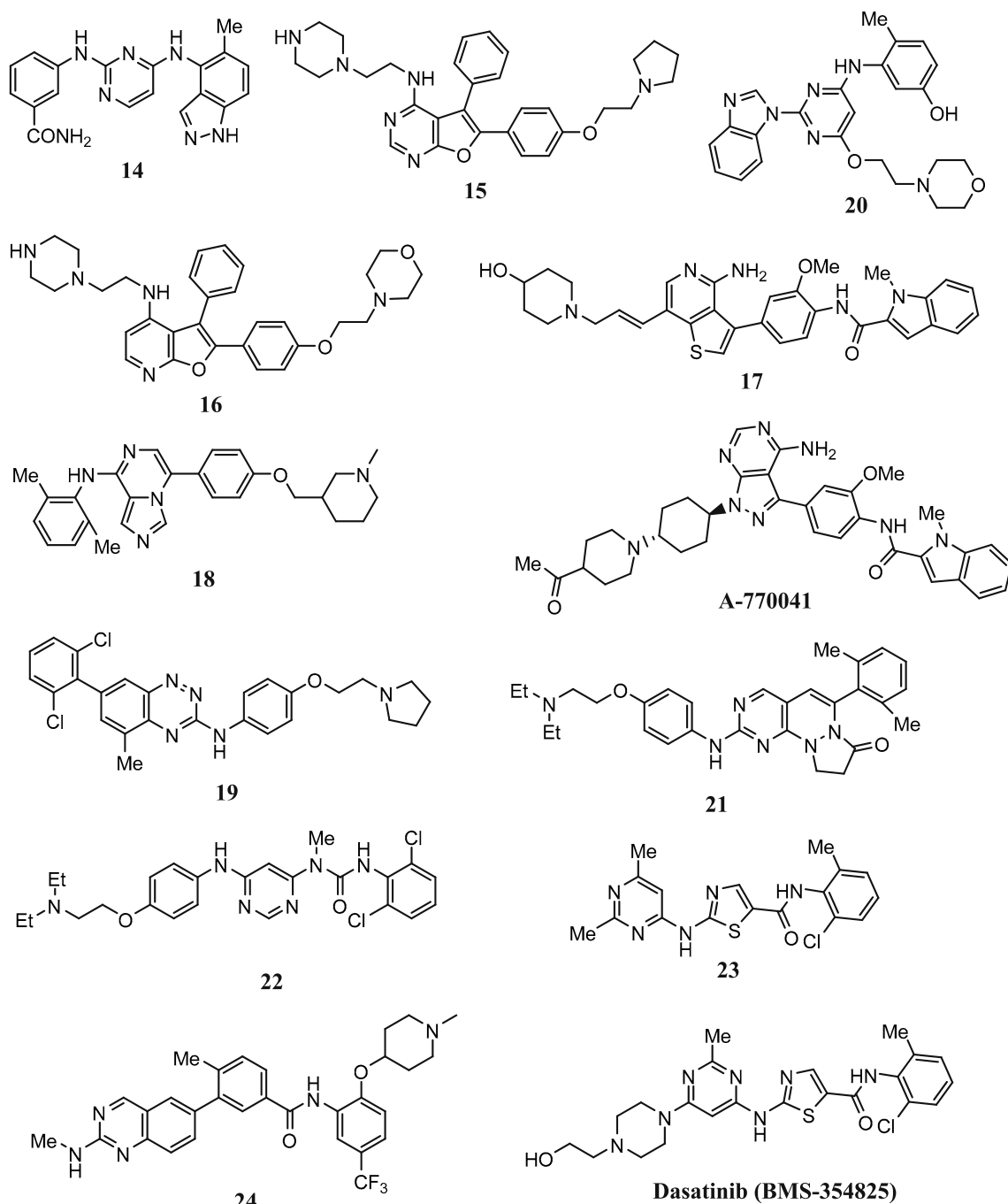
Fig. 3 Lck inhibitors

JAK3 inhibitors

The Janus kinases, JAK1, JAK2, JAK3, and Tyk2, are cytoplasmic protein tyrosine kinases that play a critical role in the cytokine receptor binding-triggered signal transduction through the STAT (signal transducers and activators of transcription) proteins [45]. Binding of cytokines activates the JAK kinases which phosphorylate and activate the STAT proteins. The STAT proteins form homo- or hetero-dimers and translocate to the nucleus where they induce

transcription of proinflammatory genes. JAK3 is expressed at high levels in NK cells and normally in thymocytes, platelets, mast cells, and inducible T and B cells. JAK3, which is associated with the cytokine signaling through the γc-chain of the IL-2 receptor, is critical for lymphocyte survival, differentiation, and function. In humans, mutations in JAK3 have been associated with severe combined immunodeficiency (SCID) [46] and JAK3 knockout mice are found to display defects in T, B, and NK cell development and function [47]. Therefore, inhibition of

JAK3 has potential applications in the treatment of inflammation, allergy, autoimmune disorders, and organ transplant rejection [45]. A number of JAK3 inhibitors, such as WHI-P131, WHI-P154, and PNU156804, which are not highly selective against other members of the JAK family of kinases, have been reported and included in a review article [48]. This review will focus on JAK3 inhibitors reported during 2006–2007 and the references cited here refer to the inhibitors reported earlier. A number of JAK3 inhibitors have been disclosed in an abstract, manuscript, or at scientific meetings without disclosing their structure and/or pharmacology profile (for example, R348, an inhibitor of JAK3 and Syk [49]); such inhibitors are not covered in this review.

A selective JAK2 inhibitor could have a potential anti-inflammatory effect through the inhibition of the Th1 pathway. However, the reported and available JAK2 inhibitors have some degree of JAK3 inhibitory activity and therefore the observed effect could, at least partly, be due to concomitant JAK3 inhibition. This review will not include the JAK2 inhibitors that are reported to have JAK3 inhibitory activity.

Figure 4 shows the structure of JAK3 inhibitors discussed below. PF-956980, a structurally close analog of CP-690550, has been reported to be a potent and selective inhibitor of JAK3 with $IC_{50}=4$ nM (vs 1 nM for CP-690550) [50]. In the human whole blood assay, the anti-CD3/CD28 antibody-stimulated production of IFN-γ was inhibited by PF-956980 with $IC_{50}=121$ nM, while CP-690550 had $IC_{50}=25$ nM. The lower potency of PF-956980 in this assay was attributed to its higher protein binding. In

a DTH test in mice, PF-956980 when dosed by an i.v. infusion inhibited the sheep red blood cell-induced paw swelling with $EC_{50}=5$ mg/kg.

CP-690550, a potent JAK3 inhibitor with in vitro enzyme inhibitory and cellular activity as described above, is found to inhibit JAK2 kinase significantly [51]. The compound is found to exhibit profound immunosuppressive activity in a variety of animal models [52, 53]. In a CIA model in mice, a 5 mg/kg per day oral dose of CP-690550 was well tolerated and completely suppressed the clinical score and severity of arthritis [53]. This compound is reported to be efficacious in phase II trials in arthritis and kidney transplantation [54, 55]. In a phase II study in patients with rheumatoid arthritis, treatment with CP-690550 at an oral dose of 15 mg b.i.d. for 6 weeks resulted in 54% of the patients responding with an ACR50 score [54]. The compound was not as well tolerated at a 30 mg b.i.d. dose for 6 weeks.

A pyrrolopyrimidine series of inhibitors have been reported to be inhibitors of JAK3 [56]. Compound 25, for example, inhibited JAK3 with $IC_{50}=142$ nM and IL-4-induced TF-1 cell proliferation with $IC_{50}=140$ nM. The selectivity of this series of compounds over JAK2 was modest at best in the enzyme as well as cell assays. A series of pyrimidines with a similar activity and selectivity profile has been reported [57]. Compound 26 inhibited JAK3 with $IC_{50}=45$ nM and inhibited IL-4-induced proliferation of TF-1 cells with $IC_{50}=90$ nM. A staurosporine analog, 27, inhibited JAK3 with $IC_{50}=31$ nM [58]. This series of compounds lacked a desirable solubility profile and additional data were not disclosed.
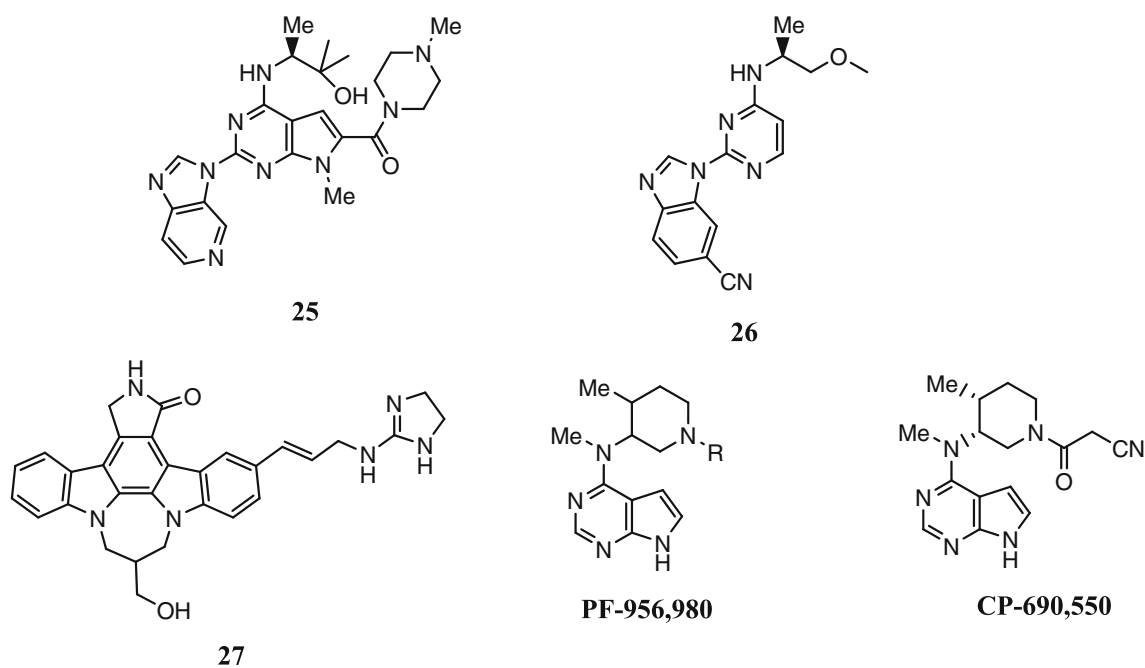

Fig. 4 JAK3 inhibitors

Concluding remarks

Discovery of kinase inhibitors for the treatment of inflammation and autoimmune disorders has been ongoing for almost two decades now. Drugs targeting p38 kinase for the treatment of arthritis and other autoimmune diseases have progressed to phase III clinical trials, but have not been found to be suitable for filing for registration. A number of drugs targeting the kinases p38, JNK, MEK, IKK2, JAK3, Lck, and Syk are currently undergoing clinical trials for the treatment of diseases related to inflammation and autoimmunity. It is anticipated that some of these or newer ones will be found suitable for the treatment of rheumatoid arthritis, psoriasis, organ transplantation, or other immune disorders.

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