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REVIEW ARTICLE

An Update on JAK Inhibitors

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Abstract: Janus kinases (JAKs) are a family of non-receptor tyrosine kinases, composed by four members, JAK1, JAK2, JAK3 and TYK2. JAKs are involved in different inflammatory and autoimmune diseases, as well as in malignancies, through the activation of the JAK/STAT signalling pathway. Furthermore, the V617F mutation in JAK2 was identified in patients affected by myeloproliferative neoplasms. This knowledge prompted researchers from academia and pharmaceutical companies to investigate this field in order to discover small molecule JAK inhibitors. These efforts recently afforded to the market approval of four JAK inhibitors. Despite the fact that all these drugs are pyrrolo[2,3-*d*]pyrimidine derivatives, many compounds endowed with different heterocyclic scaffolds have been reported in the literature as selective or multi-JAK inhibitors, and a number of them is currently being evaluated in clinical trials. In this review we will report many representative compounds that have been published in articles or patents in the last five years (period 2013-2017). The inhibitors will be classified on the basis of their chemical structure, focusing, when possible, on their structure activity relationships, selectivity and biological activity. For every class of derivatives, compounds disclosed before 2013 that have entered clinical trials will also be briefly reported, to underline the importance of a particular chemical scaffold in the search for new inhibitors.

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1. INTRODUCTION

Janus kinases (JAKs) constitute one of the recognized families of non-receptor tyrosine kinases. It is composed by four members in mammals: JAK1, JAK2, JAK3 and Tyrosine kinase 2 (TYK2). JAK1, JAK2 and TYK2 are ubiquitously expressed, while JAK3 is mainly localized in hematopoietic cells [1]. These kinases transmit signals from cell membrane receptors to members of the signal transducer and activator of transcription (STAT) family. The membrane receptor ligands that activate the JAK/STAT signalling pathway are constituted by a variety of cytokines, interferons, interleukins (ILs), growth factors and hormones [2]. Each JAK enzyme signals through specific STAT members (STAT 1-6) [3]. JAKs are constituted by about 1100 amino acids organized in seven homology (H)domains, JH7-JH1, from the N- terminus to the C-terminus. These domains have been successively grouped into four functional domains: a FERM domain (JH7-JH6) formed by about 350 amino acids and targeting the proteins to the plasmatic membrane; a short SH2 domain (JH5-JH3), so called for its similarity with Src-homology-2 domain; a pseudokinase domain (JH2), structurally similar to a kinase domain, but lacking the catalytic activity, and involved in the regulation

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of the catalytic domain; a catalytically active tyrosine kinase domain (JH1) [4] (Fig. **1**).

JAKs are constitutively linked to the cytoplasmic portion of cytokine or other factor receptors. Upon binding of the specific ligand to its membrane receptor, the receptor oligomerizes. These types of receptors lack intrinsic kinase activity, but their oligomerization activate JAKs, that in turn autophosphorylate and/or transphosphorylate their own JH1 domains. Following this activation, the JH1 domain phosphorylates the cytoplasmic tail of the cytokine receptor, that attracts STATs. Then, JAKs phosporylate STATs, that form activated dimers. In this form, STATs migrate to the cell nucleus and regulate the transcription of target genes [1] (Fig. **2**).

The JAK/STAT signalling pathway is activated in inflammatory diseases and in some malignancies. The role of JAKs in cytokine signalling pathways was identified in the 1990s [5], and indicated the involvement of JAKs in autoimmune or inflammatory diseases.

In 2005, the V617F mutation in JAK2 was identified in patients affected by myeloproliferative neoplasms (MPN) that include myelofibrosis, polycitemia vera and primary thrombocytopenia [6]. More recently, other JAK2 alterations, including rare exon 12 JAK2 mutations, have been identified. Hyperactivation of the JAK/STAT pathway, with or without mutations, has been detected in some solid cancers, including breast, head and neck, and lung cancers and in hematological malignancies, including multiple myeloma, Hodgking and non-Hodgking lymphomas and in acute leukemias [7].

These and other findings, extensively reported elsewhere [2, 3], prompted the research for JAK inhibitors, as potential drugs useful for the treatment of inflammatory or neoplastic diseases dependent on JAK/STAT pathway activation.

2. JAK INHIBITORS

All JAK inhibitors are ATP competitive and bind to the active conformation of the catalytic site, behaving as type I inhibitors. The majority of compounds acts through a reversible inhibition, but some irreversible inhibitors have been discovered and are here reported. The compounds show a variable grade of selectivity towards JAK1, JAK2, JAK3 and TYK2, but generally they inhibit at least two of these enzymes with nanomolar potency. As a recent general trend, multitargeted inhibitors have been shown to possess an increased activity compared with selective inhibitors. Among JAK inhibitors, the lack of selectivity for a specific family members can cause some side effects, in particular anaemia, thrombocytopaenia and increased infection risk [8, 9], but they are generally manageable with clinical surveillance and dose variations [10]. On the other hand, the search for inhibitors selective for a specific JAK family member is very active, since a selective compound could show a reduction of such undesired effects. It is important to note that JAK3, differently from the other family members that have a wide spectrum of actions in many tissue, is specifically involved in the development of immuno-competent cells [8]. For this reason, selective JAK3 inhibitors could be potential drugs for immunosuppressive and anti-inflammatory treatment. Moreover, selective isoenzyme inhibitors could serve as probes to investigate specific functions of every single JAK member.

Up to date, four JAK inhibitors entered the market. They are the pyrrolo[2,3-*d*]pyrimidines ruxolitinib **1**, tofacitinib **2**, oclacitinib **3** and baricitinib **4** (Fig. **3**).

Many interesting articles on these compounds are present in the literature. Briefly, Ruxolitinib **1**, a JAK1-JAK2 inhibitor by Jakafi, Incyte/Novartis was approved in 2011 by the US FDA [11] and in 2012 also by EMA for the treatment of myelofibrosis [12].

Tofacitinib **2**, a pan-JAK inhibitor by Pfizer, was approved in 2012 by the US FDA for the treatment of patients with moderate to severe rheumatoid arthritis (RA) not treatable with methotrexate [13]. The compound was later approved in other countries and in March 2017 also in the European Union. Oclacitinib **3**, another JAK1-JAK2 inhibitor by Zoetis (formerly the animal health business unit of Pfizer) has been recently approved for veterinary use in the treatment of canine allergic dermatitis [14]. Baricitinib (INCB28050, LY3009104) **4**, is a nanomolar inhibitor of JAK1 and JAK2 by Eli Lilly, useful for the treatment of RA, atopic dermatitis and systemic lupus erythematosus. In February 2017, EMA authorized baricitinib, used as monotherapy or in combination with methotrexate, for the treatment of moderate to severe active RA in adult

Fig. (1). Schematic representation of JAK family structure.

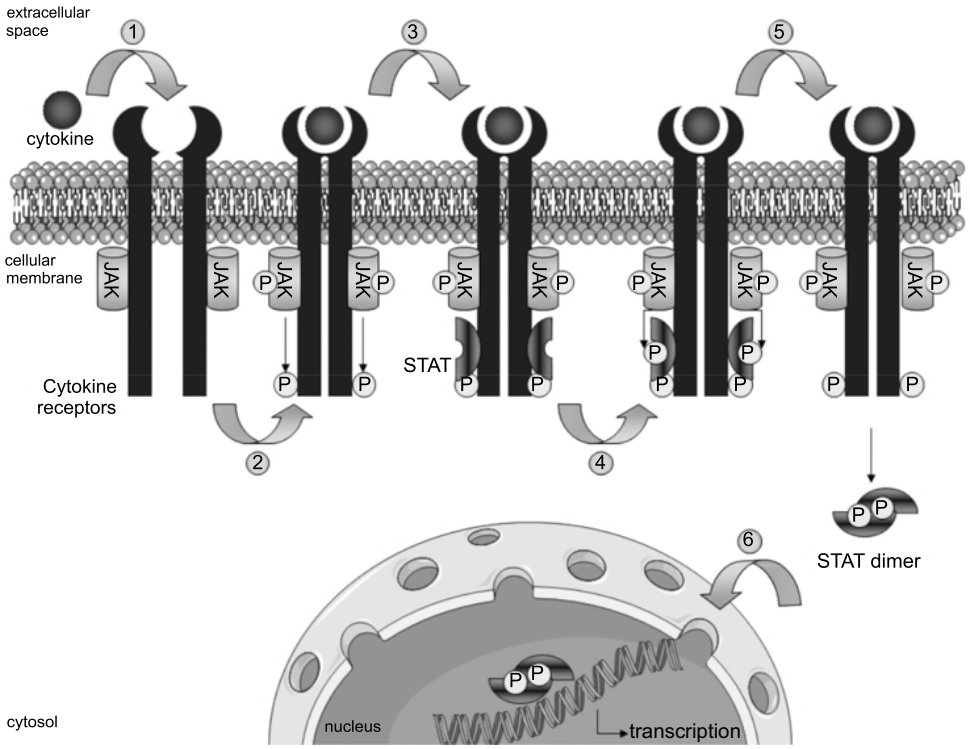

Fig. (2). The JAK/STAT signalling pathway. 1. Ligand binding. 2. Receptor oligomerization and phosphorylation (activation) of JAKs that in turn phosphorylate the cytoplasmic tail of the cytokine receptor. 3. STATs bind phosphorylated receptors. 4. JAKs phosporylate STATs. 5. STATs form activated dimers. 6. STATs migrate to the cell nucleus and regulate the transcription of target genes.

patients who can't be treated with one or more disease-modifying anti-rheumatic drugs (DMARDs). Although there have been attempts to gain approval in Japan and the USA for baricitinib as a RA treatment, the US FDA is currently unable to approve the drug, since further clinical data are needed to establish the appropriate doses and better characterize the safety profile of the compound [15].

A representative binding mode of pyrrolo[2,3-*d*] pyrimidines in the catalytic site of JAKs is reported in (Fig. **4**), showing the crystal structure of tofacitinib bound to JAK1 [16, 17]. The pyrrolo-pyrimidine core occupies the adenine pocket, forming two hydrogen bonds with the hinge region. The oxygen and nitrile groups of tofacitinib forms van der Waals contacts with several residues.

A variety of heterocyclic compounds active as JAK inhibitors is currently in preclinical or clinical trials, or have just appeared in the recent literature. In this article we report many representative compounds that have been published in articles or patents in the last five years (period 2013-2017). The inhibitors are classified on the basis of their chemical structure, focusing, when possible, on their structure activity relationship (SAR), selectivity and biological activity. For every class of derivatives, compounds disclosed before 2013 that have entered clinical trials will also be briefly reported, in order to underline the importance of a particular chemical scaffold for the search of new inhibitors. For compounds tested in clinical phases, the identification number of the trial is reported in parenthesis. Further information on every trials is available at https://clinicaltrials.gov/.

3. MONOCYCLIC COMPOUNDS

3.1. Five Membered Heterocyclic Compounds Containing One Heteroatom

Nerviano Medical Sciences synthesized a series of pyrrole-3-carboxamides active as JAK inhibitors [18]. The compounds show a slight selectivity for JAK2, but are also active on JAK1 and TYK2. One of the most active molecules is **5** (Fig. **5**) which possesses IC_{50} values of 1, 7 and 3 nM on JAK2, JAK1 and TYK2, respectively. All the compounds of the patent are active in the low micromolar-nanomolar range as antiproliferative agents on the JAK2 dependent human megakaryoblastic leukaemia cell line SET-2, while they are almost inactive on the JAK2 independent human chronic myelogenous leukaemia cell line K562. Further studies performed by the company led to the identification of the

orally available compound NMS-P953 **6** (Fig. **5**), which first appeared in a 2012 patent [19]. It has an IC_{50} value of 8 nM on JAK2, with a JAK1/JAK2 selectivity of 39. The compound shows antitumor activity when tested in the JAK2-driven SET2 xenograft model, and favourable pharmacokinetic and safety profiles [20].

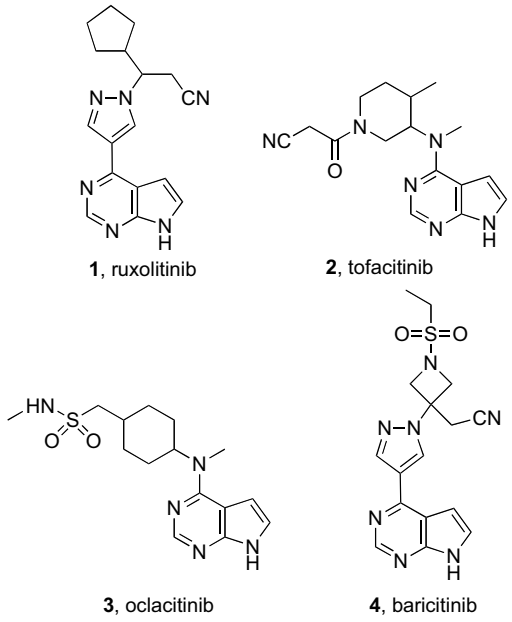

Fig. (3). Structures of marketed JAK inhibitors.

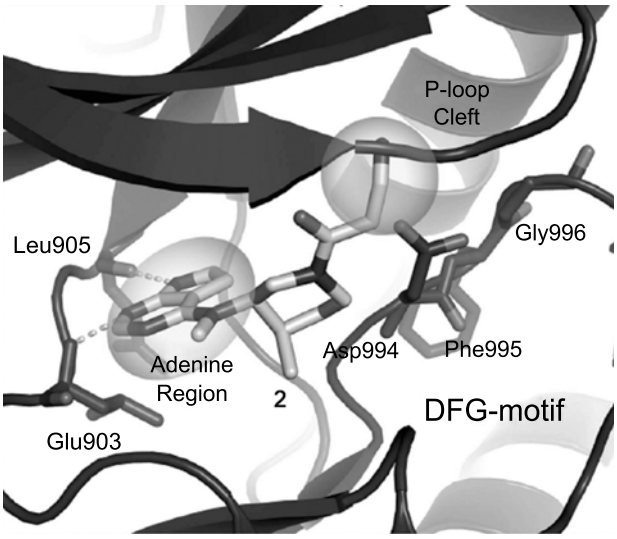

Fig. (4). Binding mode of compound **2** (tofacitinib) within the ATP-binding site of JAK1 (PDB code: 3FUP). Hydrogen bonds are represented as dashes. The most important residues are labeled and shown as sticks [17].

3.2. Five Membered Heterocyclic Compounds Containing Two Heteroatoms

In 2013, Merk Sharp & Dohme Corporation disclosed in four patents many pyrazolo-4-carboxamides, all bearing a cyano-group on the N1 substituent, active as JAK inhibitors.

All the compounds are claimed to be useful in the treatment of JAK-mediated diseases, including RA, asthma, COPD (chronic obstructive pulmonary disease) and cancer. They are more active on JAK1 than on JAK2.

The compounds of the first patent bear a substituted cycloalkylnitrile ring in N1 [21]. One of the most interesting inhibitors is the carbamate **7** (Fig. **5**), as a mixture of different diastereoisomers (1R,3S,4S and 1S,3R,4R or 1S,3S,4S and 1R,3R,4R), which shows IC_{50} values of 0.8 and 22 nM on JAK1 and JAK2, respectively. In the products of the second patent [22] the nitrogen atom of the carbamate group on the cyclohexane ring has been included in a piperidine ring, and this generally results in compounds that are slightly less active than the first patent inhibitors. Instead, in some inhibitors of the same patent, the carbamate group has been removed and very active compounds have been obtained. As an example, compound **8** (3R,4S or 3S,4R) (Fig. **5**) bearing a 4-cyanotetrahydro-2*H*-pyran-3-yl substituent on N1 of the pyrazole ring, is one of the most potent derivatives of the patent, with IC_{50} values of 0.09 and 0.7 nM on JAK1 and JAK2, respectively. In the third patent [23] the company disclosed pyrazolo-derivatives decorated in N1 with a piperidino or a cyclohexylamino group that always bear a cyanomethyl substituent on the carbon atom linking the pyrazole ring. The compounds are generally more active than those reported in the two previous patents. The most potent compound is derivative **9** (Fig. **5**), which possesses sub-nanomolar inhibition values (JAK1 IC_{50} = 0.01 nM, JAK2 IC_{50} = 0.6 nM). The compounds of the fourth patent are cyanoethylpyrazole carboxamides with activity values similar to the compounds of the first patent. As an example, derivative **10** (Fig. **5**) inhibits JAK1 and JAK2 with IC_{50} values of 1 and 13 nM, respectively [24].

In 2014 Incyte Corporation patented a series of bipyrazole derivatives [25]. The compounds share the (3-pyrazol-1-yl-azetidin-3-yl)-acetonitrile function with baricitinib, a drug previously reported. Differently from baricitinib, which inhibits JAK1 and JAK2 with a similar potency, these inhibitors are more active on JAK1 than on JAK2. One of the most active compounds is **11** (Fig. **5**), which inhibits JAK1 with an IC_{50} value lower than 300 nM and JAK2 with an IC_{50} value higher than 700 nM; however, the exact inhibitory values are not reported in the patent. The inhibitors have been tested in different cell and animal models, including a

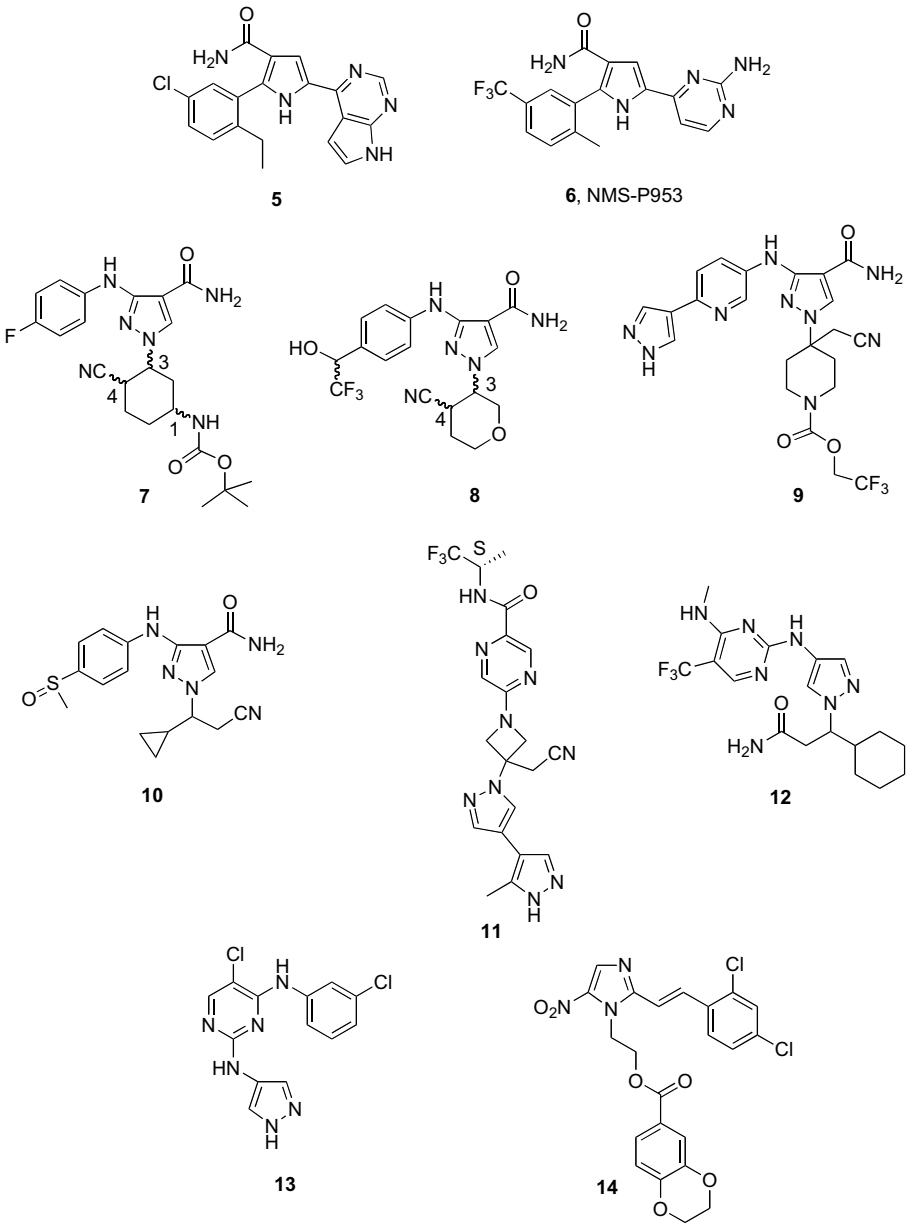

Fig. (5). Five membered heterocyclic compound JAK inhibitors.

xenograft model obtained from the INA-6 plasmacytoma cell line inoculated in SCID (severe combined immunodeficiency) mice, different models of arthritis and other autoimmune diseases, uveitis and conjunctivitis and in the murine skin contact delayed hypersensitivity response test.

Liang and colleagues reported in two articles some pyrazole compounds rationally designed as a molecular simplification of ruxolitinib. Derivative **12** (Fig. **5**) [26] inhibits JAK1, JAK2 and JAK3 with IC_{50} values of 0.67, 0.098 and 0.039 μM, respectively, in enzymatic assays and also inhibits JAK2 phosphorylation in HeLa cells. Successively, the same authors prepared other 4-amino-pyrazole derivatives not substituted in N1 [27]. Among these compounds, the most active is **13** (Fig. **5**), which possesses IC_{50} values in the range 2.2-3.5 nM on JAK1, JAK2 and JAK3. Moreover, this inhibitor displays antiproliferative activity against different cancer cell lines (PC-3, HEL, K562, MCF-7, and MOLT4) at low micromolar levels, and generally results more active than ruxolitinib. Molecular modelling studies have been performed to explain the higher activity of these N1 unsubstituted derivatives compared with the previously reported compounds, such as **12**. The authors established that the -NH moiety of the pyrazole ring forms an hydrogen bond with Glu930, and this could be very important for the improved binding of **13** compared to **12**.

A family of metronidazole derivatives has been reported as JAK3 inhibitors by Sang and colleagues. De-

rivative **14** (Fig. **5**) has an IC_{50} value of 9 nM on JAK3 and a nanomolar antiproliferative activity on different tumor cell lines, including HeLa, U251, HepG-2 and A549. Docking studies, performed by using the JAK3 crystal structure (PDB code: 3FUP), detected the binding mode of the compound to the enzyme. However, no selectivity information towards other JAK family members is reported in the article [28].

3.3. Six Membered Heterocyclic Compounds

The most important six membered heterocyclic JAK inhibitors are pyrimidine derivatives.

These types of compounds include a number of derivatives that were published in 2007-2008 and have entered clinical trials. Even if these inhibitors fall outside the topic of this article and have been extensively reported elsewhere, it is useful here to mention the most active compounds. Derivative **15**, XL019 (Fig. **6**) [29] has been evaluated in clinical trials for the treatment of myelofibrosis (NCT00522574) and polycythemia vera (NCT00595829). Compound **16**, fedratinib (Fig. **6**) [30], has been investigated in several clinical trials, including a phase II study for the treatment of myelofibrosis in patients previously treated with ruxolitinib (NCT01523171). Compound **17**, momelotinib (Fig. **6**) [31], entered in clinical trials for the treatment of myelofibrosis or post-polycythemia vera (NCT01423058, NCT01969838, NCT01998828), of metastatic pancreatic ductal adenocarcinoma in combination with other anticancer drugs (NCT02244489, NCT02101021) and of metastatic KRAS-mutated non-small cell lung cancer (NCT02258607). Unforunately, no result is available for any of these studies.

In 2016, YM Biosciences patented a series of 2-amino-pyrimidine derivatives active on all members of the JAK family [32]. The derivatives are claimed to be useful in the treatment of immunological and inflammatory diseases and of cancer. Derivative **18** (Fig. **6**) is one of the most active inhibitors, with IC_{50} values ranging between 7.40 and 22.37 nM on JAK1, JAK2, JAK3 and TYK2 in enzymatic assays. Some compounds, including **18**, tested in cell assays, inhibit STAT-5 phosphorylation. Moreover they result active when tested in a murine model of JAK2-V617F-positive myeloproliferative disease, and also show an inhibitory effect on tumor initiation, progression and metastasis formation, evaluated *in vivo* in different xenograft models in immuno-deficient mice. Some derivatives have also been evaluated in models of asthma, RA and inflammatory bowel diseases (IBD).

A class of 2,4-diamino-pyrimidines has been disclosed [33] as JAK inhibitors. The compounds are characterized by the presence of a 5-methyl-1*H*-pyrazol-3-ylamino group in C4 and of a chlorine atom in C5 of the pyrimidine ring, as well as by the presence of a 3-oxopropanenitrile group on the terminal portion of the C2 amino group side chain. The compounds are active on JAK1 and JAK2. As an example, **19** (Fig. **6**) inhibits JAK1 with an IC_{50} value of 20 nM, and JAK2 with an IC_{50} of 2 nM, while it is less active on JAK3 (IC_{50} = 118 nM).

Dana Faber Cancer Institute researchers reported a series of 2,6-diamino-5-chloro-pyrimidines active as JAK3 inhibitors [34]. The most active derivatives, including compound **20** (Fig. **6**), are irreversible inhibitors. Indeed, the acrylamide function forms a covalent bond, *via* a Michael addition, with the unique cysteine residue (Cys909) in JAK3 (not present in the catalytic site of the other JAK family members). The approach of targeting cysteine residues with an electrophilic, such as an acrylamide function, has led to successful kinase inhibitors afatinib (an EGFR inhibitor) [35] and ibrutinib (a Btk inhibitor) [36] which have been recently approved as anticancer drugs. A cocrystal structure of JAK3 in complex with **20** confirms the covalent binding of the inhibitor. The chlorine atom in C5 of the pyrimidine core seems to be responsible for potency and selectivity by interacting with Met902 of JAK3. Inhibitor **20** possesses IC_{50} values of less than 0.5 nM on JAK3 and of 896 and 1052 nM on JAK1 and JAK2, respectively, and an IC_{50} value of 20 nM on JAK3 transformed Ba/F3 cells [37].

In two 2015 patents the Spanish company Almirall disclosed two families of 5-fluoropyrimidines, bearing differently substituted amino groups in C4 and in C6, one of which is a 3-oxo-3-piperidin-1-yl-propanenitrile moiety. A fluorine atom in C5 and different groups in C2 are present. An example of the first patent is **21** (Fig. **6**) [38], while an example of the second patent is **22** (Fig. **6**) [39]. These derivatives inhibit JAK1, JAK2 and JAK3 with IC_{50} values lower than 100 μM.

In the following year, the same company disclosed other pyrimidine derivatives [40]. The compounds generally bear a polar substituent on C6 of the pyrimidine ring. They are active on the three enzymatic isoforms, even if the activity can slightly change, depending on the substitution pattern. For example, derivative **23** (Fig. **6**) is more active on JAK1 and JAK3 (IC_{50} of 0.5 and 0.7 nM, respectively) than on JAK2 (IC_{50} of 32 nM). In the three Almirall patents, different associations with other therapeutic agents and different

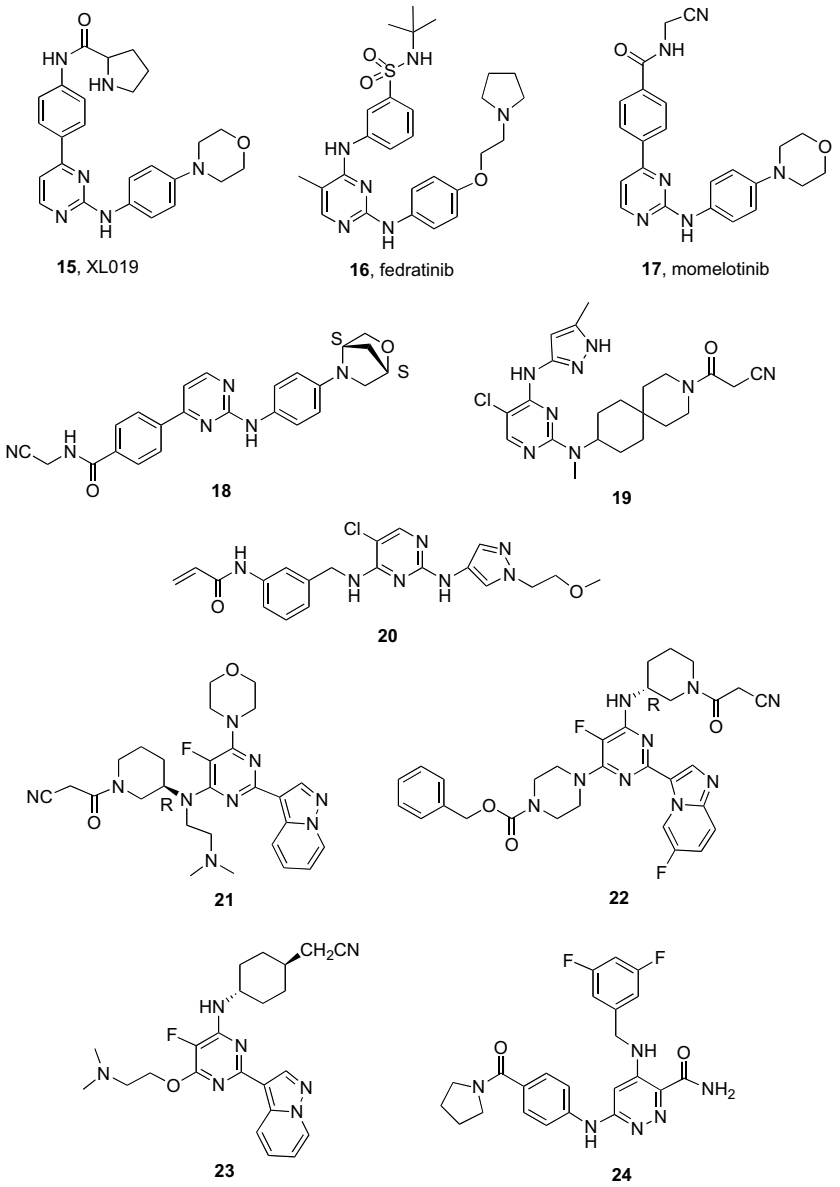

Fig. (6). Six membered heterocyclic compound JAK inhibitors.

pharmaceutical formulations have also been reported. Among six membered heterocyclic derivatives, there is a family of pyridazine-3-carboxamide derivatives bearing two amino substituents in C4 and C6, patented by Portola Pharmaceuticals as JAK inhibitors [41]. The compounds are generally more active on JAK3 (with a potency in the subnanomolar range) than on JAK1, JAK2 and TYK2. An example of such compounds is **24** (Fig. **6**).

4. BICYCLIC COMPOUNDS

4.1. Pyrrolo-fused Heterocyclic Compounds

Many JAK inhibitors, included the derivatives which entered the market, belongs to this wide family of compounds.

4.1.1. Pyrrolo[2,3-b]pyridines

Decernotinib **25** (Fig. **7**), by Vertex Pharmaceuticals [42] and peficitinib **26** (Fig. **7**), by Astellas Pharma INC [43] are two potent JAK inhibitors that are especially active on JAK3. They appeared in two 2007 patents, and their preclinical activities have been extensively reported elsewhere.

In a 2015 article, Vertex researchers described the study that led to the identification of decernotinib [44]. The lead optimization process was based on the evaluation of enzymatic data, crystallographic studies, activity in cell assays and on the efficacy in pharmacologic models of aberrant immune function, such as the rat host *versus* graft model. High potency together with an optimal pharmacokinetic profile have led the compound to clinical evaluation. Both decernotinib and

peficitinib have reached advanced phase clinical trials for the treatment of RA [45].

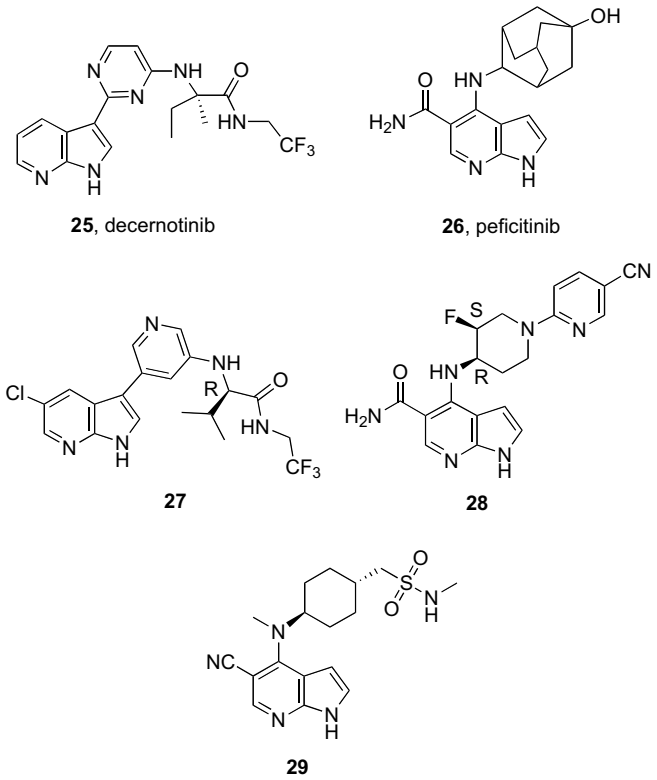

Fig. (7). Pyrrolo[2,3-*b*]pyridine JAK inhibitors.

In 2013 Merck Sharp & Dohme patented 1*H*-pyrrolo[2,3-*b*]pyridines bearing different substituents in C3 [46]. The compounds are generally more active on JAK3 than on JAK2, while they haven't be tested on JAK1. An example is derivative **27** (Fig. **7**), which shows, as the majority of the compounds of the patent, a JAK3 inhibition value less than 10 nM on JAK3 and in the range 10-500 nM on JAK2.

Two families of 4-amino-1*H*-pyrrolo[2,3-*b*]pyridines appeared in the literature in 2015. Compound **28** (Fig. **7**) is the most interesting member of a series of 5-carboxamides derivatives published by Astellas researchers [47] and first reported in a 2008 patent [48]. This compound inhibits JAK3, JAK1 and JAK2 with IC_{50} values of 0.30, 4.1 and 3.2 nM, respectively, with a weak activity on hERG (ether-a-go-go-related gene), whose inhibition is related to QT interval prolongation and to a potential cardiotoxicity. In cell assays, **28** inhibits the proliferation of IL-2-stimulated T cell, and *in vivo* prolongs graft survival in a cardiac transplant model. Moreover, orally administered, it possesses good pharmacokinetic properties in different animal species.

Compound **29** (Fig. **7**), by Novartis, is one of the most active derivatives of the other family of 2015 JAK inhibitors. It belongs to a series of derivatives of oclacitinib **3**, all characterized, such as this last, by an aminosulfonylmethylcyclohexane group and active on JAK1 [49]. These compounds could have the same use of oclacitinib in the treatment of canine skin diseases, including atopic dermatitis and pruritus. Inhibitor **29** possesses IC_{50} values of 34 and 840 nM on JAK1 and JAK3, respectively, and of 3 μM on TYK2.

4.1.2. Pyrrolo[2,3-d]pyrimidines

Ruxolitinib **1**, tofacitinib **2**, oclacitinib **3** and baricitinib **4**, the four marketed JAK inhibitors reported in the Introduction, belong to this family of compounds. Another derivative similar to baricitinib is itacitinib, INCB039110 **30** (Fig. **8**), again by Incyte [50], which is a potent JAK1 inhibitor currently being tested in thirteen phase I/II clinical trials for the treatment of inflammatory and autoimmune diseases and solid tumor and B-cell malignancies, alone or in combination with other drugs [51]. Recently, the results of a randomized, double-blind, placebo-controlled, dose-escalation study of the safety and efficacy of itacitinib indicated that the compound produced significant improvements in patients with stable, chronic plaque psoriasis [52]. Recently, results from a phase II open-label trial in patients with intermediate- or high-risk myelofibrosis indicated that the treatment with the compound can provide effective relief of myelofibrosis-related symptoms, with limited hematologic toxicity, even if the compound is less effective in reducing spleen size (splenomegaly is a common symptom of myelofibrosis) than ruxolitinib and other JAK2 inhibitors tested in phase III clinical trials [53].

In two 2013 patents, Incyte disclosed other derivatives similar to itacitinib. The compounds of the first patent, such as **31** (Fig. **8**) [54], bear a cyclohexyl azetidine moiety connected to a 4-substituted-2-trifluoromethyl-pyrimidine ring by an oxygen atom. The derivative of the second patent, such as **32** (Fig. **8**), are very similar [55], but are characterized by a piperidinylcylobutyl moiety. Compared with the previous patent compounds the nitrogen atom has been "moved" from the four-atom ring to the six-atom ring. The compounds of both patents show a similar inhibitory profile, with IC_{50} values less than 20 nM on JAK1 and a good selectivity towards JAK2 (JAK2/JAK1 IC_{50} ratio > 10). Many biological assays have been proposed for these inhibitors. They can be tested on cancer cell lines dependent on JAK/STAT signalling and on cells bearing the JAK2-V617F mutation or on T-cells.

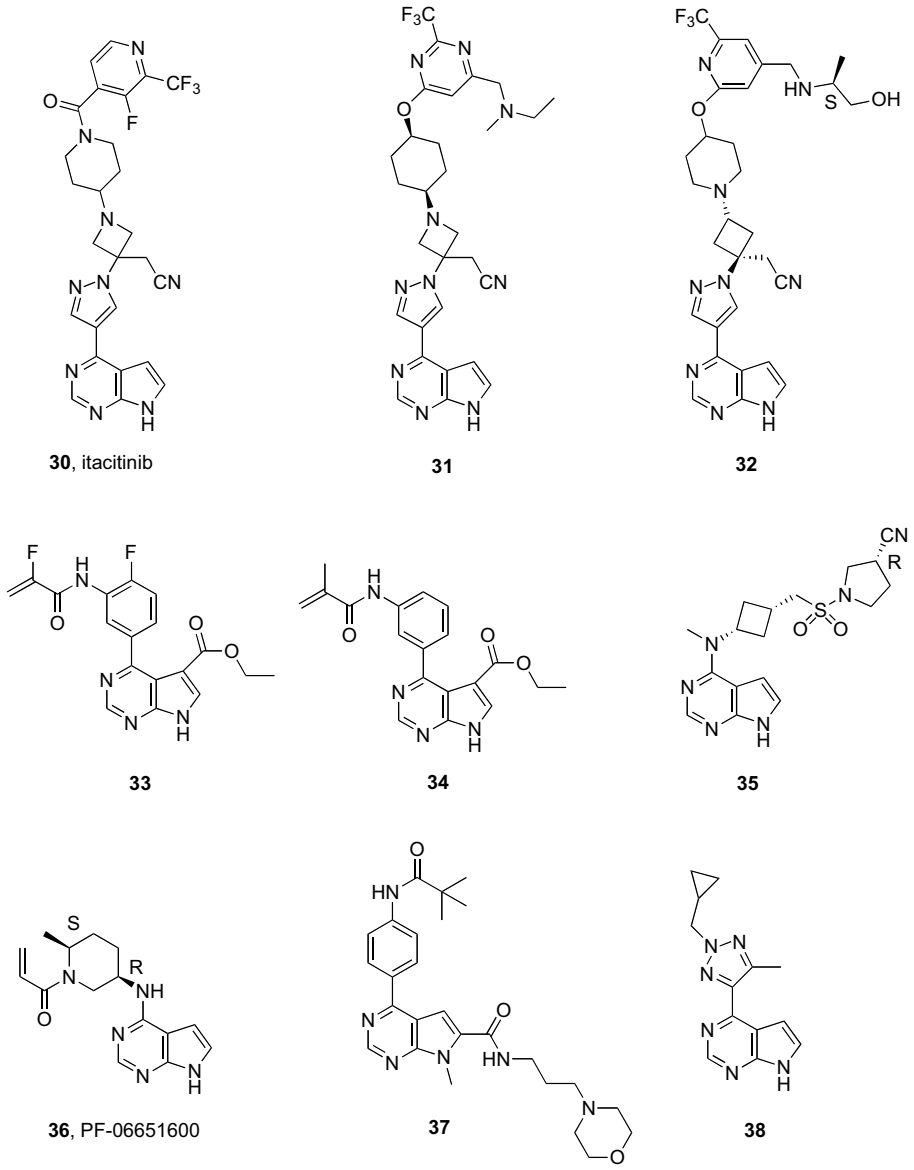

Fig. (8). Pyrrolo[2,3-*d*]pyrimidine JAK inhibitors.

Moreover, the compounds can be evaluated in human tumor xenograft models in SCID mice, in the murine skin contact delayed hypersensitivity response test, in *in vivo* anti-inflammatory tests (especially in models of arthritis) and in *in vivo* models of ocular diseases. Unfortunately, no data have been reported for cell and animal assays [54, 55].

Again in 2013, Merck patented a family of pyrrolopyrimidine as JAK3 inhibitors [56]. The majority of the derivatives are characterized by an ester function in C5 and an aryl group in C4. Compound **33** (Fig. **8**) inhibits JAK3 with an IC_{50} value of 13 nM, and JAK2 with an IC_{50} value of about 1.5 μM. Derivative **34** (Fig. **8**), present in the same patent, has been further investigated [57]. Analogously to compound **20** by the Dana Faber Cancer Institute, **34** is an irreversible inhibitor containing an electrophilic acrilamide terminal function able to react with the -SH group of Cys909 of JAK3 *via* a Michael addiction. This type of binding has been confirmed by studies on the X-ray crystal structure of **34** bound to JAK3. This molecule potently inhibits JAK3, with an IC_{50} value of 0.15 nM, and is 4300-fold selective for JAK3 over JAK1 in enzymatic assays. *In vivo*, **34** blocks the development of inflammation in a rat model of RA, while sparing hematopoiesis.

Pfizer Inc. patented many pyrrolo[2,3-*d*]pyrimidines as JAK inhibitors, including tofacitinib **2** and oclacitinib **3** [17]. More recently the company disclosed compounds in which the six-membered ring of **2** and **3** has been substituted by a four-membered ring [58]. The new compounds are more active on JAK1 than on JAK2, JAK3 and TYK2. As an example, compound **35** (Fig. **8**) inhibits JAK1 with an IC_{50} value of 5 nM, while it shows IC_{50} values of 179, 5270 and 444 nM on

JAK2, JAK3 and TYK2, respectively. These inhibitors demonstrate antiproliferative activity on T-cells in an *in vitro* assay.

In 2015 and 2016, the same company disclosed in two patents differently fused pyrrolo-derivatives, including pyrrolo[2,3-*d*]pyrimidines, as JAK3 irreversible covalent inhibitors [59, 60]. All the compounds bear an acrylamide function. The most interesting compound of the two patents is **36**, PF-06651600 (Fig. **8**) which has an IC_{50} value of 0.3 nM on JAK3 [57] and of 1640 nM on JAK1, while it is almost inactive on JAK2 and TYK2 (IC_{50} >10000 nM) [61]. PF-06651600 inhibits Th1 and Th17 cell differentiation and function *in vitro*, and it is active in the rat adjuvant-induced arthritis model and in a mouse model of autoimmune encephalomyelitis. Importantly, by sparing JAK1 function, PF-06651600 preserves JAK1-dependent anti-inflammatory signalling pathways [62]. In a recent article, the same researchers described the lead optimization process that has led to the discovery of this selective JAK3 inhibitor. They succeeded in coupling potency with a low glutatione S-transferase mediated clearance in such compound, by exploiting a distant steric effect of the methyl group inserted on the piperidine ring. The compound is metabolically stable, has optimal ADME properties and is suitable for oral use. Importantly, it has a very low reactivity for other protein containing a cysteine residue analogous to Cys909. It has been used to investigate JAK3 mediated pathways [61]. PF-06651600 it is currently being investigated in three phase II clinical trials aimed at evaluating its activity in patients with alopecia areata, RA and ulcerative colitis, while other three phase I clinical trials are terminated [63].

In 2016, Chinese researchers reported some pyrrolo[2,3-*d*]pyrimidines as JAK2 inhibitors [64]. The compounds have been designed through a hybridization strategy between the structure of ruxolitinib **1** with a pharmacophore of JAK2 inhibitors, previously identified by the same authors. The most potent compound obtained following this way is **37** (Fig. **8**), which possesses an IC_{50} value of 6 nM on JAK2 and a high selectivity towards the other isoenzymes. It also has a potent antiproliferative activity (0.14 μM) on TF-1 cells and a good pharmacokinetics profile in rats.

In the same year, South-Korea researchers disclosed 4-(2,5-triazole)-pyrrolo[2,3-*d*]pyrimidines as selective JAK2 inhibitors [65]. SAR studies indicated that the triazole ring disubstituted in N2 and C5 generally led to JAK2 selective inhibitors in this family of compounds. The most interesting derivative is **38** (Fig. **8**), with an IC_{50} value of 41.9 nM on JAK2 and a selectivity of 10.6 folds toward JAK1 and of 58.1 towards JAK3. It also inhibits the JAK2-V617F mutant. In cell assays it displays antiproliferative activity on HEL 92.1.7 cells (derived from MPN hematopoietic cancer and carrying the V617F mutant) and on NSCLC HCC827 cells, that are gefitinib resistant.

4.1.3. Pyrrolo[2,3-b]pyrazines

In this chemical class the majority of compounds are JAK3 inhibitors.

In 2013, Hoffmann-La Roche researchers published two series of 7-amido-pyrrolo-pyrazines as selective JAK3 inhibitors. Compound **39** (Fig. **9**), belonging to the first series on compounds, shows IC_{50} values of 0.26, 0.80 and 3.2 nM on JAK3, JAK2 and JAK1, respectively [66]. Importantly, this compound has been evaluated in cell models, where it demonstrates a functional selectivity for modulation of a JAK3/JAK1-dependent IL-2 stimulated pathway over a JAK1/JAK2/TYK2-dependent IL-6 stimulated pathway. Consistently, the activity of **39** is also present in *in vivo* tests. Indeed, an acute pharmacokinetics/pharmacodynamic mouse model, that was developed by the authors to confirm the inhibition of JAK family members *in vivo*, demonstrated that **39** is selective for JAK3.

The compounds belonging to the second series of 7-amido-pyrrolo-pyrazines JAK3 inhibitors bear 2-phenyl-ether substituents [67]. The authors, on the basis of molecular modelling studies, established the importance of the 2-aryl substituent to achieve selectivity towards JAK3. In fact, this aromatic moiety forms a lipophilic interaction with Cys909 of JAK3 and allows a backbone interaction with Leu828. One of the most interesting compounds is **40** (Fig. **9**), which presents IC_{50} values of 5, 39 and 262 nM on JAK3, JAK2 and JAK1, respectively (selectivity JAK2/JAK3 = 7.1, selectivity JAK1/JAK3 = 47.4) and a good activity, even if lower than that of tofacitinib, in cell assay.

In 2014, the same company published a family of JAK3 inhibitors endowed with enzymatic and cell activities comparable to those of tofacitinib. As an example, compound **41** (Fig. **9**) inhibits JAK3 with a good selectivity towards the other isoenzymes [68].

In a Pfizer patent already cited in the paragraph on pyrrolo[2,3-*d*]pyrimidines, the company also reported many pyrrolo[2,3-*b*]pyrazines as JAK3 inhibitors. These compounds bear an acrylamide substituent on the C2 substituent of the pyrazine ring and,

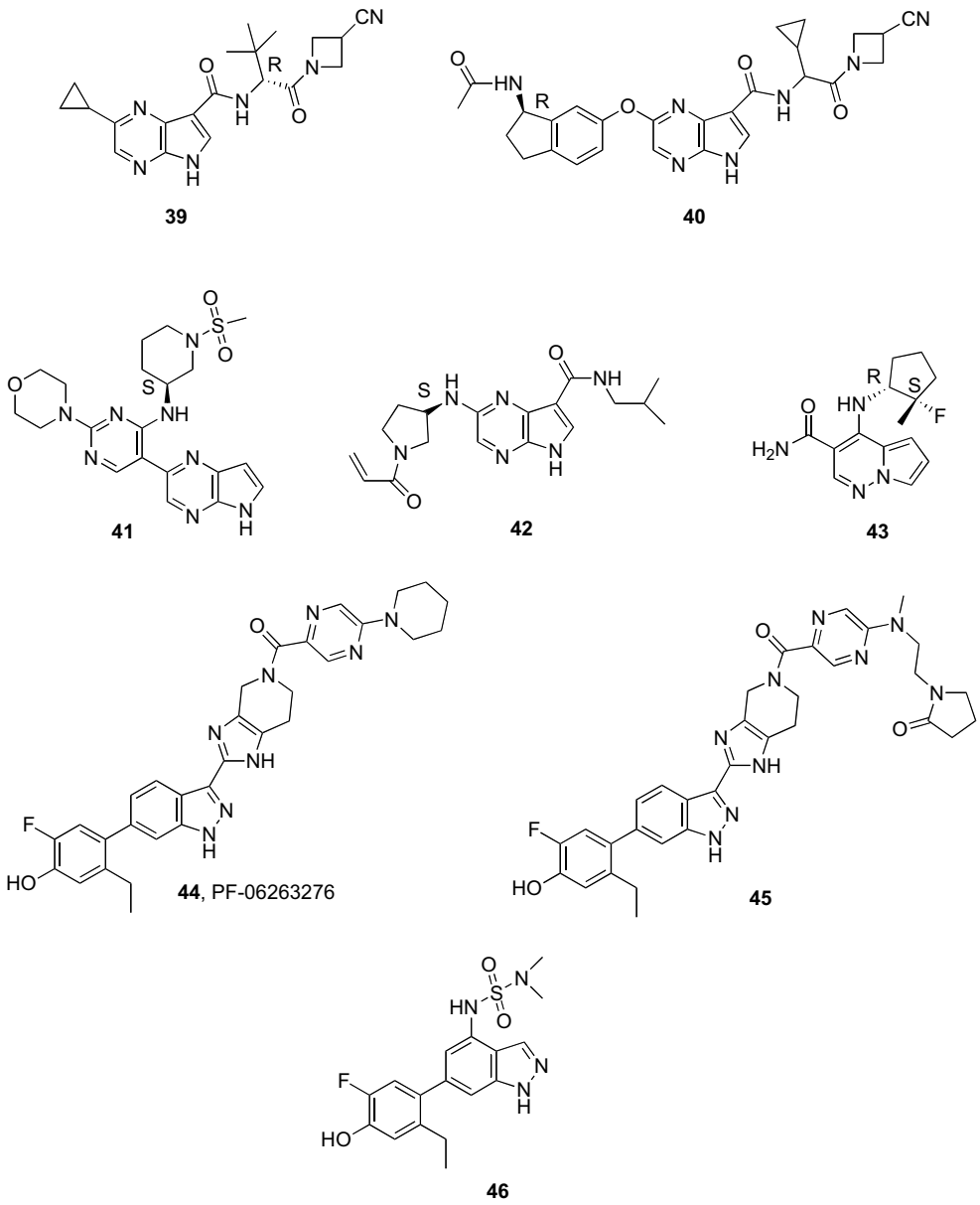

Fig. (9). Pyrrolo[2,3-*b*]pyrazine, pyrrolo[1,2-*b*]pyridazine, and 1*H*-indazole JAK inhibitors.

consequently, they behave as irreversible inhibitors of JAK3, forming a covalent bond with JAK3 Cys909 [60]. Generally, these inhibitors show nano- or sub-nanomolar IC_{50} values in enzymatic assays on JAK3 and micro- or sub-micromolar values in cell assays evaluating the ability of the compounds to reduce IL-15 induced STAT-5 phosphorylation. One of the most potent derivatives is **42** (Fig. **9**), with an IC_{50} value of 0.124 nM in enzymatic tests.

4.1.4. Pyrrolo[1,2-b]pyridazines

In 2014, Bristol-Myers Squibb published an article dealing with pyrrolo[1,2-*b*]pyridazine-3-carboxamides as JAK inhibitors [69]. The compounds, already appeared in a 2012 patent [70], are characterized by the presence of a substituted cyclopentane ring in C4. The most interesting compound is **43** (Fig. **9**), that shows IC_{50} values of 1, 9, 2, 18 towards JAK3, JAK1, TYK2 and JAK2, respectively. A X-ray structure of JAK3 complexed with **43** reveals the presence of two hydrogen bonds: one between the N1 nitrogen atom of the pyrrolo-pyridazine scaffold with Leu905 NH, and the second between the 3-carboxamide NH and the Glu903 carbonyl group. Moreover, the carbonyl of the 3-carboxamide group of the inhibitor is engaged in another hydrogen bond with Asp967 of the DFG motif (a key amino acid sequence in the activation loop), through a water molecule. The cyclopentyl substituent fits in the ribose pocket and several van der Waals interaction, that are maximized for the 1R,2S enantiomer, stabilize the interaction of the compound with the enzyme. Compound **43** possesses good pharmacokinetic

properties, is orally available, and could be an useful tool for the evaluation the dual JAK3/TYK2 inhibition *in vivo*.

4.2. Pyrazolo-fused Compounds

4.2.1. 1H-indazoles

In the last few years some indazole derivatives have been published as JAK inhibitors.

Pfizer researchers disclosed a series of indazole JAK inhibitors bearing different substituents in C3, and a phenol moiety in C6 [71] useful for the treatment of COPD. The most interesting compound of the patent is **44**, PF-06263276 (Fig. **9**), whose identification, binding mode and biological activities have been described in a recent article [72]. The crystal structure of **44** bound with JAK2 shows that it binds the kinase with a type 1.5 binding mode. In this binding mode, the DFG motif maintains the typical type-1 "in" conformation, while the gatekeeper methionine residue moves from the known orientation, allowing the formation of a deep interaction within an appropriate hydrophobic pocket. This particular binding mode allows slow-offset kinetics and an extended duration of action in cells. Compound **44** shows IC_{50} values of 2.2, 23.1, 59.9 and 29.7 nM on JAK1, JAK2, JAK3 and TYK2, respectively, and of 70.3 nM in PBMCs (pheripheral blood mononuclear cells). This compound demonstrated a favourable metabolism and pharmacokinetic properties, and was further investigated. *In vivo* tests displayed that it is active in a mouse model of IL-6 induced pSTAT response in the lung, after intratracheal administration, and in a dermal model of IL-23 induced inflammation after topical application. The authors also demonstrated that the anti-inflammatory activity of the compound is due to its local activity (in lung and skin) and not to a systemic effect. The study indicated PF-06263276 as a potential drug for inhaled or topical therapy for the treatment of COPD or psoriasis. Importantly, its local application can avoid potential side effects caused by systemic administration of JAK inhibitors. PF-06263276 has been evaluated in two phase I clinical trials (NCT01981681, NCT02193815), one for the treatment of psoriasis.

Very similar compounds have been recently disclosed in two patents by the UK company Topivert Pharma. The inhibitors, such as **45** (Fig. **9**), are claimed to be more active than PF-06263276, used as a reference compound, especially as JAK2 inhibitors [73, 74].

Leo Pharma researchers started a study to discover other JAK inhibitors for the treatment of psoriasis [75]. They applied a structure-based design on an indazole hit compound and, by modulating the substitution on the C4 sulphonamide moiety and adding the same phenol substituent present in C6 of compound **44**, they obtained quite active pan-JAK inhibitors, such as compound **46** (Fig. **9**). It shows pIC_{50} values of 8.36, 8.2 and 8.5 on JAK1, JAK2 and JAK3, respectively, and of 7.0 on the B lymphocyte Ramos cell line expressing STAT-6. Unfortunately, **46** and its congeners have poor light stability and show phototoxicity, probably due to the presence of the indazole scaffold.

4.2.2. Pyrazolo[4,3-c]pyridines

In 2013, Cellzome published two patents dealing with 1*H*-pyrazolo[4,3-*c*]pyridine JAK3 inhibitors. The compounds of the first patent are characterized by a 2-fluoro (or 2,5-difluoro)-benzyl group in N1 and by a substituted pyrazol-3-ylamine moiety in C6 [76]. One of the most potent inhibitors is **47** (Fig. **10**), showing an IC_{50} value lower than 0.1 μM on JAK3 and higher than 10 μM on JAK2 in enzymatic assays. Since STAT-5 phosphorylation is one of the proximal events in the signalling pathways downstream JAK3, the compounds of this patent have been tested on human YT cells (a NK-like cell line), in which stimulation with IL-2 causes STAT-5 phosphorylation. Compound **47** displays an inhibition value lower than 10 μM in this cell assay.

The second patent compounds maintain the same substituted benzyl group in N1 and bear a pyridin-2-ylamine moiety in C6. Generally, these molecules are slightly less active than the just reported compounds [77]. However some derivatives, such as the nicotinamide derivative **48** (Fig. **10**), have inhibitory potency similar to that of the first patent molecules, both in enzymatic and in cell assays.

Different series of pyrazolo[4,3-*c*]pyridinones have been reported as potent JAK inhibitors by Merck Sharp & Dohme in some patents. The compounds are characterized by common C3 substitutions, while the decoration in N1 varies in the different families of derivatives. The first patent inhibitors, such as **49** (Fig. **10**), are substituted by a cyclohexanecarbonitrile moiety in N1 [78] and are quite selective for JAK1. For example, compound **49** inhibits JAK1 and JAK2 with IC_{50} values of 0.039 and 0.19 nM, respectively. Compound **50** (Fig. **10**) and its congeners, reported in the second patent, bear a cyanoethyl chain linked in N1 [79]. This substitution is already present (even if on other heterocyclic scaffolds) in baricitinib and itacitinib. The compounds are generally less selective towards JAK1 than

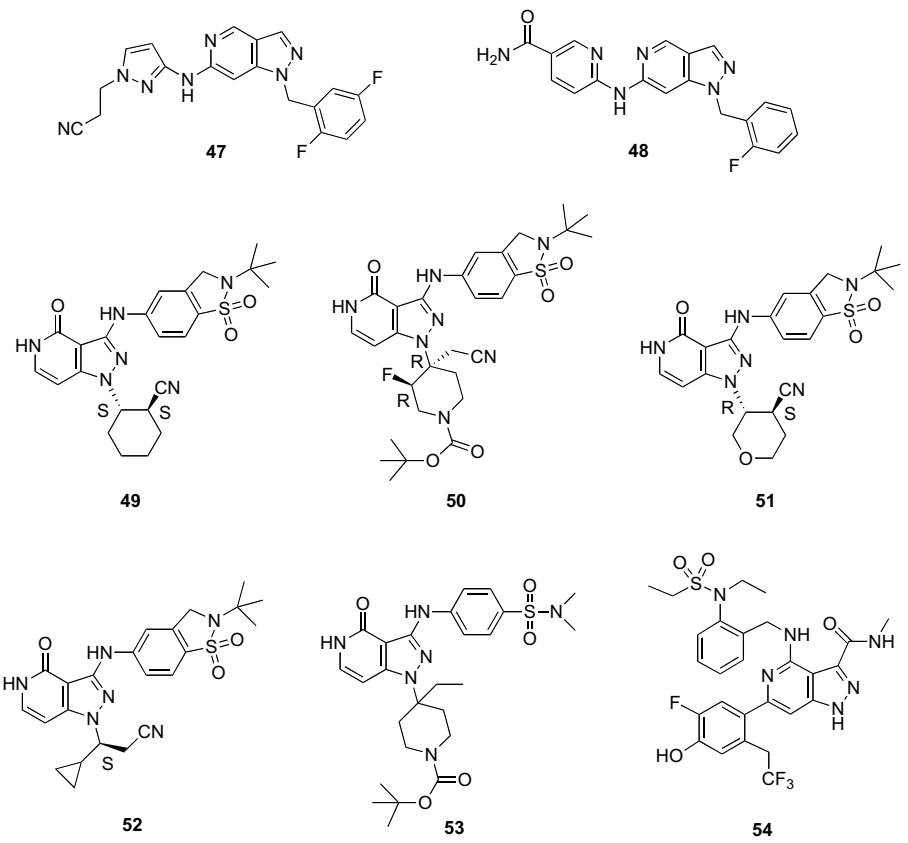

Fig. (10). Pyrazolo[4,3-*c*]pyridine JAK inhibitors.

the compounds of the first patent. As an example, **50** shows a similar inhibitory activity on JAK1 and JAK2 (IC_{50} values of 0.15 and 0.19 nM, respectively).

The compounds of the third patent are very similar to **49**, both in the chemical structure and in the inhibitory profile [80]. In such derivatives the cyclohexane ring of **49** has been generally substituted by a tetrahydropyrane ring. An example is **51** (Fig. **10**), possessing IC_{50} values of 0.05 nM on JAK1 and of 0.15 nM on JAK2.

The fourth patent compounds, such as **52** (Fig. **10**), bear a cyclopropyl ring on the cyanoethyl N1 chain and maintain the same activity of the just reported inhibitors **50** and **51** [81].

Two 2016 patents of the same company disclosed other JAK inhibitors [82, 83]. The compounds do not bear a nitrile group as the derivatives of the 2014 patents. In fact, an ethyl chain on C4 of the piperidine ring and a Boc group on the N1 of the same ring are typical of these derivatives, that generally are slightly less active than the cyano derivatives and do not show JAK1/JAK2 selectivity. Derivative **53** (Fig. **10**), present in both patents, inhibits JAK1 with an IC_{50} of 0.20 nM and JAK2 with an IC_{50} of 0.40 nM.

Pfizer patented many pyrazolo-pyridines and pyrazolo-pyrimidines as JAK1 selective inhibitors [84]. One of the most interesting inhibitors is the pyrazolo[4,3-*c*]pyridine **54** (Fig. **10**), which inhibits JAK1 with an IC_{50} value of 8.0 nM, and JAK2, JAK3 and TYK2 with IC_{50} values of 38.1, 176.8 and 36.5 nM, respectively. The compound shows an IC_{50} value of 67.4 nM on A549 cell line, in which STAT-3 phosphorylation has been stimulated by recombinant human IFNγ.

4.2.3. Pyrazolo[1,5-a]pyrimidines

Differently decorated pyrazolo[1,5-*a*]pyrimidines have been patented by pharmaceutical companies in 2015.

A series of 2-amino-pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid amides are inhibitors of JAK1 and JAK2. As an example compound **55** (Fig. **11**) inhibits the two enzymes with IC_{50} values of 87 and 42 nM, respectively [85], and displays favourable pharmacokinetics after intravenous and oral administration in mice, rats, dogs and monkeys.

Celon Pharma disclosed pyrazolo[1,5-*a*]pyrimidine derivatives bearing substituents in the positions 2,3,5,7 of the scaffold [86]. The compounds are claimed to be

JAK2 inhibitors, with IC_{50} values lower than 10 nM for the most active compounds, such as **56** (Fig. **11**). No enzymatic data have been reported for the other JAK family members.

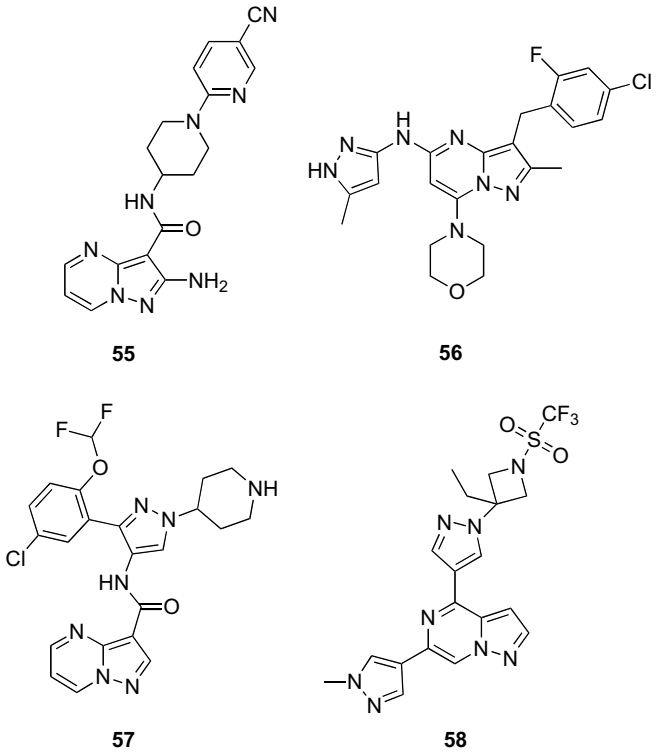

Fig. (11). Pyrazolo[1,5-*a*]pyrimidine and pyrazolo[1,5-*a*]pyrazine JAK inhibitors.

Another family of pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid amides have been patented by Hoffmann La Roche as potent JAK1 and JAK2 inhibitors with activity similar to that of the amido-derivatives of the series of compound **55**, just reported [87]. In the amidic portion, the disclosed compounds present a pyrazole ring bearing in C3 a 5-chloro-2-difluoromethoxyphenyl group. Compound **57** (Fig. **11**), belonging to this family, possesses IC_{50} values of 1.62 and 0.46 nM on JAK1 and JAK2. In a cellular assay that utilizes TF-1 human erytroleukemia cells to measure JAK1-dependent STAT-6 phosphorylation downstream of IL-13 stimulation, the compound has an EC_{50} value of 21 nM. Such JAK inhibitors have a high systemic clearance. In the treatment of pulmonary diseases, including COPD, it is advantageous to have high drug concentration in the lung with a lower concentration in the blood stream or in other organs. For this reason it is important to dispose of compounds with high systemic clearance *via* the inhaled delivery route.

4.2.4. Pyrazolo[1,5-a]pyrazines

In 2016 Array Biopharma patented 4,6-disubstituted pyrazolo[1,5-*a*]pyrazine derivatives [88]. Compound **58** (Fig. **11**), bearing in C4 a pyrazolo-sulphonamidic substituent very similar to that of baricitinib, is one of the most potent compounds of the patent, and shows IC_{50} values lower than 10 nM for JAK2 and TYK2, in the range 10-100 nM for JAK1, and higher than 1000 nM for JAK3.

4.3. Imidazolo-fused compounds

4.3.1. Benzoimidazoles

Compound **59**, CHZ868 (Fig. **12**), was developed in a collaboration among Dana Faber Cancer Institute, other US institutes and Novartis [89]. The compound is a type II JAK2 inhibitor, binding and stabilizing the inactive form of JAK2, differently from the majority of JAK2 inhibitors, that are type I inhibitors and bind to the active enzymatic form. Molecular modelling studies showed that the hydrogen bond between N3 of CHZ868 benzimidazole ring and the NH of Asp994 of the DFG motif is one of the fundamental interaction for the type II binding mode of the compound. It inhibits JAK2 with an IC_{50} value of 110 nM in an enzymatic assay. CHZ868 is active on MPN cells resistant to type I JAK inhibitors. This activity is also present in murine MPN models. In a further study, the authors demonstrated that the compound is also active in a subset of B cell acute lymphoblastic leukemias (B-ALLs) with CRLF2 rearrangements, which are dependent on JAK2 signaling and are not sensitive to type I JAK2 inhibitors. The authors also reported the synergic activity of the combination of CHZ868 with dexamethasone. In preclinical test, this combination improved *in vivo* survival compared to CHZ868 alone. This combination could be used in clinical trials, supported by the fact that association between glucocorticoids and tyrosine kinase inhibitors are being tested for the treatment of B-ALLs [90].

A group of Korean researchers identified a family of 1,2-disubstituted benzimidazole-5-carboxamides as selective JAK1 inhibitors [91]. The most interesting compound is **60** (Fig. **12**), which has an IC_{50} value of 50 nM on JAK1 and a selectivity of 63, 25 and 74 folds *versus* JAK2, JAK3 and TYK2, respectively.

Molecular modelling studies indicated that the 2-aminoethyl and piperidin-4-yl group of the compound confer the ability to preferentially bind the catalytic site of JAK1 than that of JAK2. Moreover, **60** is selective for JAK1 when tested on a panel of protein kinases. This selectivity is very important to evaluate the specific functions of JAK1 and the effects of its inhibition. This type of study is not possible with potent JAK inhibitors, such as tofacitinib which inhibits both

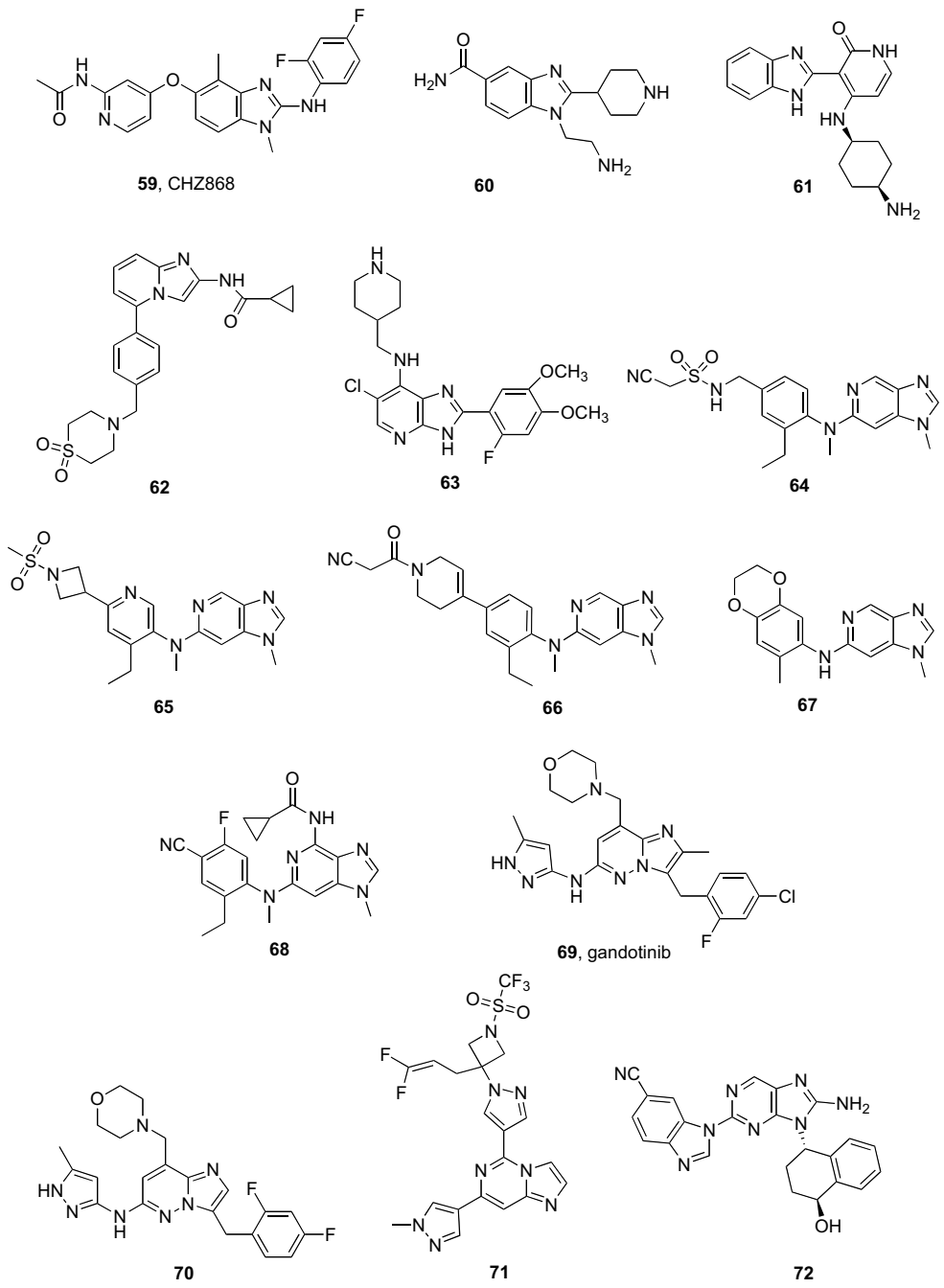

Fig. (12). Imidazole-fused derivative JAK inhibitors.

JAK1and JAK3, and for which it is not possible to attribute the therapeutic effects to the inhibition of one or the other (or both) of the kinases. Unfortunately, as outlined by the authors, compound **60** suffers from poor pharmacokinetic properties and this family of substituted benzoimidazoles needs to be optimized for a potential therapeutic use.

A collaboration among some US pharmaceutical companies led to the discovery of benzimidazole derivatives active as potent JAK1 inhibitors [92] that could lack some toxic effects, such as anemia, of the non-selective JAK1/JAK2 marketed inhibitors. The most interesting compound is **61** (Fig. **12**), obtained by means of SAR studies and crystallography-driven explorations on this family of derivatives. The compound inhibits JAK1 with an IC_{50} value of 0.1 nM, and shows a JAK2/JAK1 inhibition ratio of 40. Its selectivity is probably due to the ability of targeting some residues unique to JAK1, *i.e.* Arg879 and Glu966, as indicated by the crystal structure of **61** bound to JAK1, that was prepared by the authors. This compound is also selective when tested on a panel of 265 kinases.

4.3.2. Imidazo[1,2-a]pyridines

The Chinese company JN Therapeutics disclosed a family of substituted imidazo[1,2-*a*]pyridin-2-ylamines. Few compounds resulted selective JAK1 inhibitors [93]. Compound **62** (Fig. **12**), a deaza-analogue of the potent JAK1 inhibitor filgotinib (after reported among the fused triazole derivatives) is one of the most active derivatives. It inhibits JAK1 with an IC_{50} value lower than 500 nM, with a selectivity ratio greater than 8 for JAK1 *versus* JAK2, JAK3 and TYK2. The most active compounds have also been tested in TF-1 cells, to evaluate the JAK1 or JAK2 inhibition by measuring the cytokine-mediated phosphorylation of STAT proteins in these cells. In this assay compound **62** displays an IC_{50} value lower than 500 nM for JAK1 inhibition. Some derivatives inhibit JAK1 in human whole blood assay, are active in a collagen-induced arthritis rat model and have good pharmacokinetic properties.

4.3.3. Imidazo[4,5-b]pyridines

AstraZeneca researchers published a series of imidazo[4,5-*b*]pyridines active as JAK1 inhibitors [94]. Compound **63** (Fig. **12**), derived from SAR and molecular modelling studies, shows an IC_{50} value of 22 nM on JAK1, with 957 fold selectivity *versus* JAK2. It also inhibits STAT-3 phosporylation in NCI-H1975 cells with an IC_{50} value of 39 nM. A X-ray crystal structure of **63** bound to human JAK1 has been obtained by the authors. The compound fits the catalytic site of the enzyme, forming a pair of hydrogen bonds, one between the N atom of the pyridine ring and the NH of Leu959 of the hinge region, and the second between the NH of the imidazo-pyridine scaffold and the CO group of the same amino acid. The fluoro-substituent contributes to stabilizing these interactions. Probably, the selectivity of **63** for JAK1 *versus* JAK2 is due to the presence of Glu966 in JAK1 *versus* Asp939 in JAK2, even if, as indicated by the authors, the side chain of Glu966 is quite far from the nearest -OCH_3 group of **63**.

The compound was also tested in an *in vivo* NCI-H1975 mouse xenograft pharmacodinamic study to evaluate its activity in modulating STAT-3 phosphorylation. In this assay the inhibitor demonstrates a notable inhibition (about 80%) of pSTAT-3 formation.

4.3.4. Imidazo[4,5-c]pyridines

The Belgo-Dutch pharmaceutical company Galapagos patented many JAK inhibitors characterized by a (substituted-phenyl)-methyl-(1-methyl-1*H*-imidazo [4, 5-*c*]pyridin-6-yl)-amino scaffold. Three patents have been published in 2013. The first patent compounds bear an ethyl group on C2 and different substituents on C4 of the phenyl ring [95]. These molecules are generally active as JAK1 inhibitors. Depending on the C4 substituent, they show different activities on the other JAK family members both in enzymatic and in specific cell assays. One example is compound **64** (Fig. **12**), that inhibits JAK1, JAK3 and TYK2 with IC_{50} values in the range 0.01-100 nM. The compounds have also been evaluated in the septic shock model and in different models of arthritis, in oncology models, in IBD models, and asthma models. Aqueous solubility, plasma protein binding, microsomal stability and Caco-2 permeability have been determined for the most active compounds. One of the disclosed molecules, derivative **65** (Fig. **12**), was further developed in a 2014 patent [96]. It is a JAK1 selective inhibitor, with a K_i value of 9.21 nM on JAK1 and at least ten fold less active on the other JAK family members. The compound results active in different *in vitro* and *in vivo* models of inflammatory pathologies and JAK-driven myeloproliferative diseases.

The second 2013 patent is devoted to compound **66** (Fig. **12**) (already present in WO 2013117645 [95]) [97] and its *in vitro* and *in vivo* biological activity [98]. The compound is a potent JAK1 inhibitor, with selectivity values of 28, 30 and 9 *versus* JAK2, JAK3 and TYK2, respectively. It can be useful for the treatment of allergic, inflammatory and autoimmune diseases, proliferative diseases and cartilage pathologies.

In the third patent similar compounds have been patented, mainly as JAK1 and/or JAK2 inhibitors, depending on the substitution pattern on the phenyl ring [99]. As an example, compound **67** (Fig. **12**) inhibits JAK1 in an IC_{50} range 0.01-100 nM, while it inhibits JAK2 with IC_{50} values in the micromolar range and is not active on JAK3.

In 2017 the same company patented another family of imidazo[4,5-*c*]pyridines, which, differently from the just reported compounds, are substituted in C4 generally with an amido moiety [100]. The compounds are particularly active on JAK1 and TYK2, but some of them also inhibit JAK2 with IC_{50} values in the low nanomolar range. One of the most potent JAK1 inhibitors is **68** (Fig. **12**) which has an IC_{50} value of 1 nM on this enzyme. Analogously to the other disclosed compounds, these inhibitors have been tested in *in vitro* and *in vivo* models of inflammatory diseases and malignancies involving JAK activation, including lung cancer cell lines bearing JAK1 mutations and mice engrafted tumors containing JAK1 mutations.

4.3.5. Imidazo[1,2-b]pyridazines

Gandotinib, LY2784544, **69** (Fig. **12**) is an orally active JAK2 inhibitor disclosed by Eli-Lilly in a 2010 patent [101]. Successively the company got further insight into this derivative. It displays an IC_{50} value of 2 nM for JAK2 and of 8 nM for JAK3. The high resolution crystal structure of **69** with JAK2 demonstrates that the compound binds to the catalytic site of the enzyme. The chlorine atom on the para-position of the phenyl ring fits near Gly993. The presence of this chlorine atom explains the selectivity of the compound for JAK2 over JAK3. In fact, in JAK3, Ala966, which has the same relative position as Gly993 in JAK2, forms a more sterically-demanding ATP-binding pocket that is unable to profitably interact with the Cl atom of **69**.

The selectivity for JAK2 over JAK3 was confirmed using cell-based assays monitoring STAT-5 phosphorylation. The compound is particularly potent in inhibiting JAK2-V617F stimulated cell pathways. Its activity on this JAK2 mutation is particularly significant, due to the prevalence of the JAK2-V617F mutation in MPN and its constitutive activity. The compound is active in *in vivo* models, such as the JAK2-V617F murine ascitic tumor and a mouse JAK2-V617F hematologic disease model. Interestingly, the compound does not show any effect on erytroid progenitor cells that express wild-type JAK2. Consistently, the authors observed that the compound is poorly active on wild-type JAK2-dependent models. This inhibitor may result very useful in the treatment of JAK2-V617F-induced MPN pathogenesis with a minimal effect on normal progenitor cells [102]. Gandotinib is being currently tested in some phase I-II clinical studies to assess its safety and activity for the treatment of MPN, polycythemia vera, essential thrombocythemia and myelofibrosis [103].

In 2014, the Polish company Celon Pharma disclosed a series of selective JAK2 inhibitors, that are structurally correlated to **69** [104]. The most active compound is **70** (Fig. **12**), possessing an IC_{50} value of 1.1 nM on JAK2. JAK2 activity was confirmed by the inhibition of STAT-3 phosphorylation in HEL-92.1.7 erythroleukemia cell line harboring the JAK2-V617F mutation. *In vivo* pharmacokinetic tests indicate a better biological behaviour of **70** compared with **69**.

4.3.6. Other Imidazole-fused Derivatives

In 2013, other imidazole-fused derivatives have been patented as JAK inhibitors.

Array Biopharma disclosed 5,7-substituted imidazo[1,2-*c*]pyrimidines possessing variable activity, but usually scarce selectivity, on the different JAK family members [105]. One of the most active compounds is **71** (Fig. **12**) which shares some chemical features with baricitinib and shows IC_{50} values lower than 10 nM for JAK1, JAK2 and TYK2 and an IC_{50} value in the range 10-100 nM for JAK3 in enzymatic assays.

Ligand Pharmaceuticals patented different heterocyclic compounds, including purine derivatives active as JAK3 inhibitors [106]. For example, compound **72** (Fig. **12**) has an IC_{50} value of 0.18 nM on JAK3. The most interesting inhibitors have also been tested in a cellular assay using the mouse F7 pre-B lymphocyte cell line and in an *in vivo* test to evaluate the reduction of the IL-2-induced IFNγ production in mice after the administration of the JAK3 inhibitors.

4.4. Fused Triazoles

The compounds belonging to this chemical class active as JAK inhibitors are [1,2,4]triazolo[1,5-*a*] pyridines. The most famous compound is filgotinib, GLPG0634, **73** (Fig. **13**), first appeared in a 2010 patent by Galapagos [107]. In 2014 the company described the studies that led to the discovery of this potent JAK1 inhibitor [108]. The compound inhibits JAK1 with an IC_{50} value of 10 nM, with a JAK1 selectivity index of 2.8, 81 and 11.6 *versus* JAK2, JAK3 and TYK2, respectively. It was also tested in different *in vitro* tests: in a whole blood assay it has IC_{50} values of 0.6 μM on JAK1 and of 17.5 μM on JAK2. To investigate the binding mode of filgotinib, the compound was cocrystallized with the JH1 domain of JAK2. It binds to the ATP binding site of the enzyme. The triazolopyridine structure interacts with the hinge region of the catalytic domain by forming two hydrogen bonds, one between the N3 of the triazolo moiety and the NH group of Leu932, the second between the exocyclic NH of the compound and the CO group of Leu932. The phenyl ring forms hydrophobic interactions. The thiomorpholine dioxide moiety interacts with the glycine rich loop (a catalytic domain motif, which locates the ATP for γ-phosphate transfer to the substrate, and for this reason also known as P-loop), the side chains of Val863, Lys882 and Asp994 (of the DFG motif in the catalytic site). The compound was also tested *in vivo* in the rat collagen-induced arthritis model, where a significant reduction of the clinical score was observed at low doses (0.1 and 0.3 mg/Kg). The good pharmacokinetic profile, and in particular the oral availability in different animal species, together the activity in preclinical models led to the development of this inhibitor in clinical trials [109, 110].

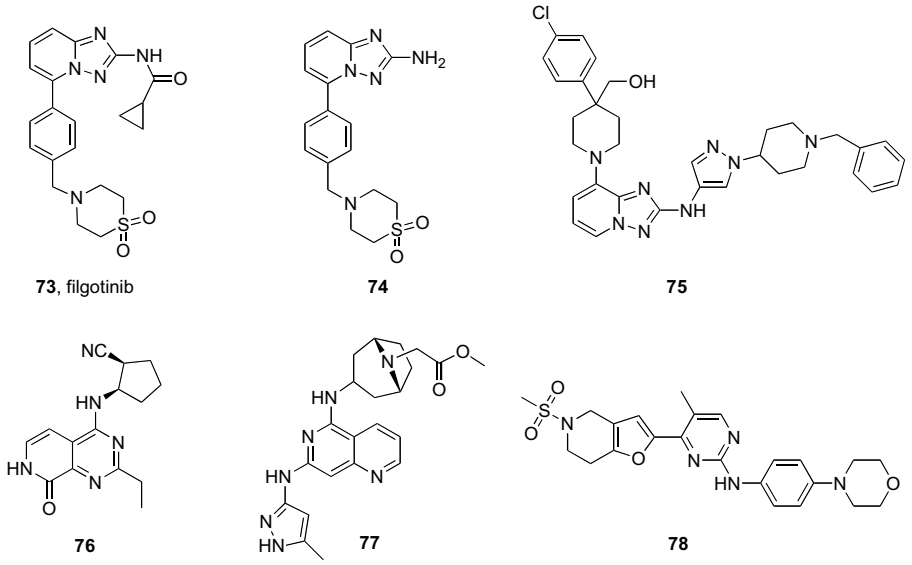

Fig. (13). Fused triazole and other bicyclic derivative JAK inhibitors.

Currently, the compound is being tested in 20 clinical trials, some of them just concluded, for the evaluation of its activity on RA, IBD and in cutaneous lupus erythematosus. The most advanced studies are phase III trials on filgotinib as a treatment option for RA, ulcerative colitis, and Crohn's disease [111].

In 2013 the same company described the filgotinib derivative **74** (Fig. **13**) [112]. The compound is less active than filgotinib, with IC_{50} values of 307 and 397 nM on JAK1 and JAK2, respectively, while it is a micromolar inhibitor of JAK3 and TYK2. In different cellular assays it displays selectivity towards JAK1. Compound **74** was tested in arthritis models where it is effective in reducing disease symptoms, but at a higher dose than filgotinib. A combination of **74** at a dose of 25 mg/Kg with filgotinib at a dose of 3 mg/Kg has a synergic effect compared with the effect of the two drugs administered alone. Interestingly, *in vivo* ADME studies indicated that **74** has a different profile in human compared with the other tested animal species. Indeed, in human the molecule has a terminal half life three time higher than in the other species. This *in vivo* behaviour offers the possibility of a low frequency dosage regimen (from once daily to once weekly).

Hoffmann-La Roche patented a series of 2,8-disubstituted [1,2,4]triazolo[1,5-*a*]pyridines, characterized by the presence of a 3-amino-pyrazolo-substituent in C2, active as JAK1/JAK2 inhibitors [113]. Compound **75** (Fig. **13**) shows a K_i value of 0.63 nM on JAK1 and of 0.38 nM on JAK2. Only enzymatic data are given in the patent.

4.5. Other Bicyclic Derivatives

In 2013, Genenthec researchers published a family of 7*H*-pyrido[3,4-*d*]pyrimidin-8-one derivatives as JAK1/JAK2 inhibitors [114]. The interactions of these inhibitors and JAK1 have been reported. In detail, the pyridone oxygen forms a hydrogen bond with the backbone NH of Leu959, and the pyridone NH forms a second hydrogen bond with the CO of Glu957 of the hinge region. Moreover, a water mediated hydrogen bond is formed between the N2 of the pyrimidine ring and the CO of Leu959. The substituent in C4 of the pyrimidine ring is in contact with the JAK1 P-loop. The modulation of the C4 substituent led to very active compounds, such as **76** (cis racemate) (Fig. **13**), which shows IC_{50} values of 3 and 1 nM on JAK1 and JAK2, respectively, and a low activity on JAK3 and TYK2. The compound is also active in IL6-pSTAT-3 and in EPO-pSTAT-5 cell models with EC_{50} values of 300 and 115 nM, respectively, and displays good ADME properties.

In 2016, Theravance Biopharma patented a series of [1,6]naphthyridine-5,7-diamines as JAK1/JAK2 inhibitors, characterized by the presence of a differently substituted 8-azabicyclo[3.2.1]oct-8-yl group on the C5 amino group and a 5-methyl-1*H*-pyrazol-3-yl on the C7 amino group [115]. Among these derivatives, compound **77** (Fig. **13**) possesses K_i values lower than 0.1 nM on JAK1 and JAK2 (while it is less active on JAK3 and TYK2) in enzymatic assays, and EC_{50} values between 31 and 200 nM in THP-1 and BEAS-2B cell assays.

The tetrahydrofuro[3,2-*c*]pyridine **78** (Fig. **13**) and its analogues were patented as JAK2 inhibitors by a Chinese company [116] and recently described in an article [117]. Compound **78** inhibits JAK2 with an IC_{50} value of 0.7 nM and a selectivity of 33 fold *versus* JAK3, resulting more active and more selective than tofacitinib.

5. TRICYCLIC COMPOUNDS

Gehringer and coll. synthesized a series of 1,6-dihydrodipyrrolo[2,3-*b*:2'3'-*d*]pyridines as JAK3 inhibitors, derived from a rigidization strategy starting from the tofacitinib structure [118]. The most active compound **79** (cis racemate) (Fig. **14**), docked into the JAK3 binding pocket (PDB code: 3LXK), revealed a binding mode similar to that of tofacitinib. **79** is a potent and selective JAK3 inhibitor, with IC_{50} values of 0.22, 3.38, 2.07 and 6.67 on JAK3, JAK1, JAK2 and TYK2, respectively. The compound has also been evaluated in a STAT phosphorylation assay in HeLa cells, using tofacitinib as a reference compound. The compound inhibits STAT-1/2/3 phosphorylation with the same potency of tofacitinib, while it is more active than tofacitinib towards STAT-5/6, with IC_{50} values lower than 3 nM. These cell studies demonstrated a different intracellular behaviour of **79** compared with that of tofacitinib and indicated that the new compound can be an useful probe to investigate the role of JAK3 inhibition.

Very recently, Astellas Pharma researchers described the biological behaviour of compound **80** (Fig. **14**) which first appeared in a 2010 patent [119]. Derivative **80**, named AS2553627, which is strictly correlated to **79**, shows IC_{50} values of 0.14, 0.46, 0.30 and 2.0 nM on JAK3, JAK1, JAK2 and TYK2, respectively, and is selective on JAK when tested on a panel of kinases. In cell assays, the compound inhibits IL-2 stimulated cells and rat T cells with IC_{50} values of 2.2 and 4.3 nM, respectively. The authors got further insight on the effect of this JAK inhibitor on cardiac rejection in rat cardiac allografts. The compound gave good results in the treatment of both acute and chronic rejection in rats, especially in combination with tacrolimus, the immunosuppressive drug usually used after organ transplant. This association decreases cardiac allograft vasculopathy and fibrosis, not inhibited by tacrolimus alone. The use of AS2553627 has a low risk for anemia in rats. The study indicates that AS2553627 could be useful in the treatment of rejection in cardiac transplantation [120].

A number of imidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridines resulted active as JAK inhibitors. The most important compound with this chemical structure is the Bristol-Myers Squibb derivative **81**, BMS-911543 (Fig. **14**), first disclosed in 2011 [121], potentially useful in the therapy of MPN. More recently its discovery and biological behaviour have been reported [122]. The compound is a potent and selective JAK2 inhibitor, with IC_{50} values of 1.1, 75, 360 and 66 nM towards JAK2, JAK3, JAK1 and TYK2, respectively, in enzymatic assays, with a high selectivity on a wide panel of kinases. The authors obtained a X-ray crystal structure of **81** bound with the kinase domain of JAK2. The unsubstituted nitrogen atom of the pyrazole ring forms a hydrogen bond with Tyr931, and the molecule has a planar disposition in the catalytic site of the enzyme. The contact between the 1,5-dimethyl-pyrazole ring and the amino acids of the JAK2 hinge region are responsible for the inhibitor selectivity *versus* the other JAK family members. In cell assays, **81** displays a potent antiproliferative activity on cell lines dependent upon JAK2 pathway, such as SET-2 and BaF3-V617F cells (IC_{50} of 80 and 65 nM, respectively), while it is poorly active on non-JAK2 dependent cell lines or on cells whose growth is dependent on other JAK family members. The compound also decreases STAT-5 phosphorylation in a mouse pharmacodynamic model and demonstrates an optimal ADMET profile. Further studies indicated that BMS-911543 has distinct inhibitory effects on STAT-5 signaling in genetically engineered mice with pancreatic cancer [123]. The compound has been tested in a phase 1/2 study (NCT01236352) to evaluate the safety, efficacy, pharmacokinetics and pharmacodynamics in subjects with myelofibrosis, but no result is yet available. A preclinical study showed that BMS-911543 has limited activity in a murine model of JAK2-V617F-driven MPN. This failure is probably due to the fact that the compound mainly targets JAK2 and this specific inhibition is not sufficient for the anticancer activity [124].

Another Bristol-Myers Squibb compound with a biological behaviour similar to that of **81** is derivative **82** (Fig. **14**), which bears a 4,5-dimethyl-thiazole ring instead of the 1,5-dimethyl-1*H*-pyrazole ring of **81** on the tricyciclic scaffold [125]. Compound **82** has IC_{50} values of 2.5, 270, 160 and 110 nM on JAK2, JAK1, JAK3 and TYK2, respectively, and an IC_{50} value of 65 nM on the SET-2 cell line.

The imidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridine **83** (Fig. **14**), by Gehringer and coll., is an aza-isoster of **79** and as the cis racemic mixture shows an IC_{50} value of 5 nM

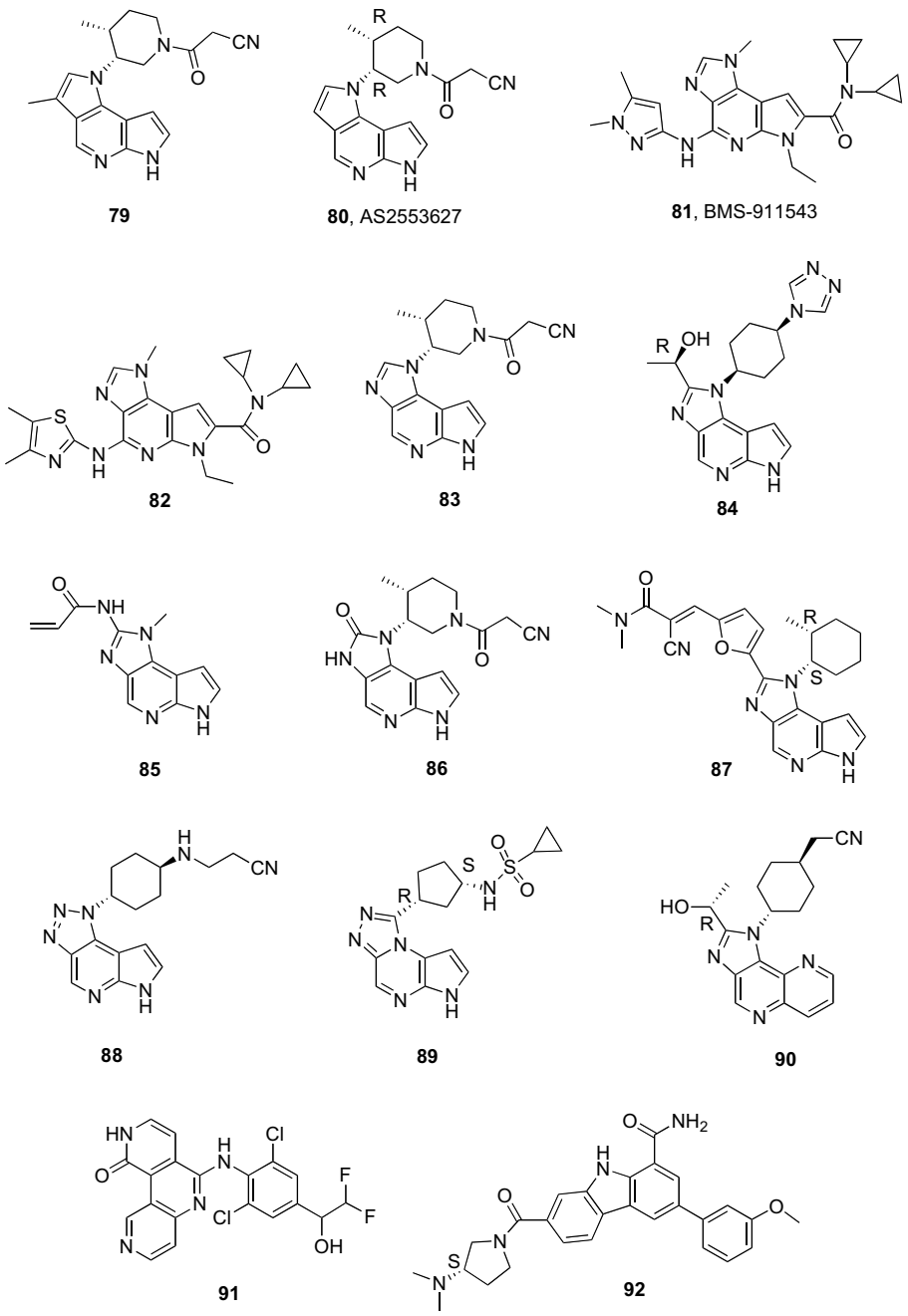

Fig. (14). Tricycle derivative JAK inhibitors.

on JAK3 in enzymatic assays. No further tests have been performed on these derivatives [126].

Other imidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridines have been patented by Hoffmann-La Roche in 2013 [127]. The compounds bear a variety of substitution on the 1 and 2 positions of the tricyclic scaffold and are generally more active on JAK1 than on the other JAK family members. For example, compound **84** (Fig. **14**) shows IC_{50} values of 9.7, 48, 21 and 24 nM on JAK1, JAK2, JAK3 and TYK2, respectively. Only enzymatic data are reported in the patent.

The AbbVie Bioresearch Center synthesized derivative **85** (Fig. **14**), with the same tricyclic structure of the just reported compounds, but bearing in C2 an acrylamide moiety able to covalently bind Cys909 in the ATP binding site of JAK3 [128]. The compound inhibits JAK3 with an IC_{50} value of less than 3 nM, while the other JAK family members are inhibited by micromolar concentrations. This derivative is selective *versus* a wide panel of kinases and inhibits JAK3 dependent pathways in different cell based tests, with IC_{50} values lower than 100 nM.

A family of 3,6-dihydro-imidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-ones has been published by Astellas researchers [129]. SAR evaluation and molecular modelling studies led to the identification of derivative **86** (as cis racemic mixture) (Fig. **14**), in which the cyanoacetamide substituent extends towards the glycine loop in the catalytic site of JAK3, contributing to the increased activity of the compound, compared to that of other molecules with the same chemical scaffold reported by the authors. The racemic mixture of **86** potently inhibits JAK3 and JAK1 with IC_{50} values of 1.1 and 1.5 nM, respectively, while it slightly less active on JAK2 (IC_{50} = 2.6 nM). The compound also shows antiproliferative activity on T-cell in the nanomolar range and a good metabolic stability.

Recently, Laufer and colleagues disclosed some derivatives active as selective JAK3 inhibitors that bind the enzymes in a covalent reversible fashion [130]. The most interesting compound is **87** (Fig. **14**), that is a picomolar JAK3 (IC_{50} = 154 pM) inhibitor with a wide kinome selectivity. Such selectivity is due to its concurrent covalent reversible binding with Cys909 and its interactions with a ligand-induced binding pocket, formed by Arg911, Asp912 and Arg953. The crystal structure of **87** in complex with JAK3 and studies of density maps, performed by the authors, demonstrated that the compound is present both covalently and non-covalently bound to the enzyme. The coexistence of these two different binding modes confers a highly reversible character of the covalent interaction. The potent enzymatic activity of **87** is also translated in cell assays. Indeed the compound selectively inhibits JAK3 signalling in human CD4 T cells.

Pyrrolo[2,3-*b*][1,2,3]triazolo[4,5-*d*]pyridines, such as **88** (Fig. **14**) are JAK1 inhibitors with a modest selectivity *versus* JAK2. In fact, **88** has IC_{50} values of 5.9 nM and 21 nM for JAK1 and JAK2, respectively, with a 3.6 selectivity for JAK1, while it is more than ten fold less potent on JAK3 and TYK2. The compound is active in pSTAT-3-IL6 JAK1 driven TF-1 cell based assay and displays a cell based selectivity for JAK1 over JAK2 of 6.7 fold (evaluated as the ratio pSTAT-5-EPO EC_{50}/pSTAT-3-IL6 EC_{50}). In rats, the compound shows a satisfying oral bioavailability, with a pharmacokinetic profile similar to that of tofactinib [131].

A family of pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazines have been reported by AbbVie Bioresearch Center researchers as quite selective JAK1 inhibitors [132]. For the development of these compounds, the authors started from the hypothesis that the induced fit of the glycine-rich loop in JAK family is critical for JAK1/JAK2 selectivity, since this loop contains at its end a His885 in JAK1 and a Asn859 in JAK2. For this reason, a selectivity between JAK1 and JAK2 could be achieved by modulating the substituent that projects under the glycine-rich loop (in this case it is the group on C3 of the triazole ring). A SAR study led to compound **89** (Fig. **14**), containing a sulphonamide moiety, already present in oclacitinib and in baricitinib, on the side chain. This compound has IC_{50} values of 0.11 and 1.9 nM on JAK1 and JAK2, respectively, together with a JAK2/JAK1 cellular selectivity of 17. It also displays a good pharmacokinetic profile, evaluated in rats. In an acute *in vivo* model of cytokine production, **89** demonstrates a good inhibition of Concanavalin A IFNγ induced production, significant as a readout of JAK1 inhibition.

Compound **90** (Fig. **14**), by Hoffmann-La Roche, is representative of a few 5:6:6 fused tricyclic compounds appeared as JAK inhibitors. It belongs to a family of imidazo[4,5-*c*]-1,5-napthyridines, bearing similar substituents of derivatives such as **84**, patented by the same company. The compounds are in general moderately selective for JAK1 towards JAK2 [133]. Derivative **90** inhibits JAK1 and JAK2 with IC_{50} values of 0.21 and 1.6 nM, respectively.

Also among 6:6:6 fused heterocyclic derivatives are present JAK inhibitors. Merck researchers published a series of pyrido[4,3-*c*]-1,6-naphtyridin-10(9*H*)-ones active selective for JAK2 [134]. Compound **91** (Fig. **14**), identified using SAR and molecular modelling investigations, shows IC_{50} values of 0.2 and 26 nM on JAK2 in enzymatic and cell assays, respectively, with a 100-fold JAK2 selectivity over 98% on a wide kinase panel. Moreover, it has a favourable pharmacokinetic profile.

Bristol-Myers Squibb published a family of carbazole-1-carboxamide derivatives as JAK2 inhibitors [135]. These compounds first appeared in a 2010 patent [136]. In particular, the (*S*)-dimethylamino-pyrrolidine amide **92** (Fig. **14**) shows IC_{50} values of 3.5, 158, 225 and 243 nM towards JAK2, JAK1, JAK3 and TYK2, respectively, in enzymatic assays, with a selectivity greater than 45-folds for JAK2 *versus* the other JAK family members, and a good selectivity when tested on a panel of kinases. Moreover it has IC_{50} values of 80 nM in a cellular assay on SET-2 cells. Regarding ADME properties of **92**, water solubility is good, but permeability (evaluated with PAMPA) and metabolic stability are not completely satisfying.

CONCLUSION

As demonstrated by the high number of chemically different JAK inhibitors synthesized by both pharmaceutical companies and academia, this type of pharmacologically active compounds represents a hot topic.

Rapid advances have been made in JAK inhibition based therapy, both in inflammatory and in MPN diseases. Currently, different JAK inhibitors are being tested in advanced clinical trials, and it is very likely that some of them will be approved on the market in the near future.

With the obtainment of more selective JAK inhibitors, safety and efficacy will probably increase. On the other hand, it should be noted that targeting more than one JAK member could be not detrimental, particularly when a pathologic state is driven by more than one cytokine. This trend has been demonstrated by the non-selective JAK inhibitors that entered in clinical use. Indeed, especially in the therapy of cancer, where many kinase pathways are involved, multikinase inhibitors have led to great successes. Also the use of combinations of JAK inhibitors with other agents could offer new therapeutic strategies, devoid of some undesired side effects. Covalent inhibitors offer a different opportunity to interact with kinases, useful for the obtainment of potential drugs, as demonstrated by the approval of axitinib, a covalent VEGFR inhibitor, and of ibrutinib, a covalent Btk inhibitor, both used in cancer therapy. Even if some in some tumor cell conditions other studies are needed [137], this field of medicinal chemistry research is in rapid evolution and it will probably lead to more active or less toxic compounds or compound associations in the next few years.

LIST OF ABBREVIATIONS

ADME	=	Absorption, distribution, metabolism, excretion, toxicity
B-ALL	=	B cell acute lymphoblastic leukemia
Btk	=	Bruton's tyrosine kinase
CD	=	Cluster of differentiation
COPD	=	Chronic obstructive pulmonary disease
CRLF2	=	Cytokine receptor like factor 2
DFG	=	Asp-Phe-Gly
DMARD	=	Disease-modifying anti-rheumatic drug
EGFR	=	Epidermal growth factor receptor
EMA	=	European Medicines Agency
FDA	=	Food and Drug Administration
HCC	=	Hepatocellular carcinoma
hERG	=	Ether-a-go-go-related gene
IBD	=	Inflammatory bowel disease
IFNγ	=	Interferon gamma
IL	=	Interleukin
JAK	=	Janus kinase
MPN	=	Myeloproliferative neoplasms
NK	=	Natural killer
NSCLC	=	Non small cell lung carcinoma
PAMPA	=	parallel artificial membrane permeability assay
PBMC	=	Pheripheral blood mononuclear cell
RA	=	Rheumatoid arthritis
SAR	=	Structure activity relationship
SCID	=	Severe combined immunodeficiency
Src	=	Sarcoma tyrosine kinase
STAT	=	Signal transducer and activator of transcription
TYK2	=	Tyrosine kinase 2
VEGFR	=	Vascular endothelial growth factor receptor

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Babon, J.J.; Lucet, I.S.; Murphy, J.M.; Nicola, N.A.; Varghese, L.N. The molecular regulation of Janus kinase (JAK) activation. *Biochem. J.*, **2014**, *462*, 1-13.

[2] Menet, C.J.; Mammoliti, O.; López-Ramos, M. Progress toward JAK1-selective inhibitors. *Future Med. Chem.*, **2015**, *7*, 203-235.

[3] Roskoski, R. Jr. Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases. *Pharmacol. Res.*, **2016**, *111*, 784-803.

[4] Yamaoka, K.; Saharinen, P.; Pesu, M.; Holt, V.E. 3rd; Silvennoinen, O.; O'Shea, J.J. The Janus kinases (Jaks). *Genome Biol.*, **2004**, *5*, 253.

[5] Darnell, J.E. Jr; Kerr, I.M.; Stark, G.R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*, **1994**, *264*, 1415-1421.

[6] Jones, A.V.; Kreil, S.; Zoi, K.; Waghorn, K.; Curtis, C.; Zhang, L.; Score, J.; Seear, R.; Chase, A.J.; Grand, F.H.; White, H.; Zoi, C.; Loukopoulos, D.; Terpos, E.; Vervessou, E.C.; Schultheis, B.; Emig, M.; Ernst, T.; Lengfelder, E.; Hehlmann, R.; Hochhaus, A.; Oscier, D.; Silver, R.T.; Reiter, A.; Cross, N.C. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood*, **2005**, *106*, 2162-2168.

[7] Quintás-Cardama, A.; Verstovsek, S. Molecular pathways: Jak/STAT pathway: mutations, inhibitors, and resistance. *Clin. Cancer Res.*, **2013**, *19*, 1933-1940.

[8] Ghoreschi, K.; Laurence, A.; O'Shea, J.J. Selectivity and therapeutic inhibition of kinases: to be or not to be? *Nat. Immunol.*, **2009**, *10*, 356-360.

[9] Ghoreschi, K.; Gadina, M. Jakpot! New small molecules in autoimmune and inflammatory diseases. *Exp. Dermatol.*, **2014**, *23*, 7-11.

[10] Saeed, I.; McLornan, D.; Harrison, C.N. Managing side effects of JAK inhibitors for myelofibrosis in clinical practice. *Expert Rev. Hematol.*, **2017**, *10*, 617-625.

[11] Deisseroth, A.; Kaminskas, E.; Grillo, J.; Chen, W.; Saber, H.; Lu, H.L.; Rothmann, M.D.; Brar, S.; Wang, J.; Garnett, C.; Bullock, J.; Burke, L.B.; Rahman, A.; Sridhara, R.; Farrell, A.; Pazdur, R. U.S. Food and Drug Administration approval: ruxolitinib for the treatment of patients with intermediate and high-risk myelofibrosis. *Clin. Cancer Res.*, **2012**, *18*, 3212-3217.

[12] Barosi, G.; Rosti, V.; Gale, R.P. Critical appraisal of the role of ruxolitinib in myeloproliferative neoplasm-associated myelofibrosis. *Onco Targets Ther.*, **2015**, *18*, 1091-1102.

[13] van Vollenhoven, R.F. Rheumatoid arthritis in 2012: Progress in RA genetics, pathology and therapy. *Nat. Rev. Rheumatol.*, **2013**, *9*, 70-72.

[14] Gonzales, A.J.; Bowman, J.W.; Fici, G.J.; Zhang, M.; Mann, D.W.; Mitton-Fry, M. Oclacitinib (APOQUEL(®)) is a novel Janus kinase inhibitor with activity against cytokines involved in allergy. *J. Vet. Pharmacol. Ther.*, **2014**, *37*, 317-324.

[15] Markham, A. Baricitinib: First global approval. *Drugs*, **2017**, *77*, 697-704.

[16] Williams, N.K.; Bamert, R.S.; Patel, O.; Wang, C.; Walden, P.M.; Wilks, A.F.; Fantino, E.; Rossjohn, J.; Lucet, I.S. Dissecting specificity in the Janus kinases: the structures of JAK-specific inhibitors complexed to the JAK1 and JAK2 protein tyrosine kinase domains. *J. Mol. Biol.*, **2009**, *387*, 219-232.

[17] Musumeci, F.; Sanna, M.; Grossi, G.; Brullo, C.; Fallacara, A.L.; Schenone, S. Pyrrolo[2,3-*d*]pyrimidines as kinase inhibitors. *Curr. Med. Chem.*, **2017**, *24*, 2059-2085.

[18] Nerviano Medical Sciences S.R.L. Substituted pyrroles as kinase inhibitors and their preparation. WO 2014019908, **2014**.

[19] Nerviano Medical Sciences S.R.L. Preparation of pyrimidinylpyrrole derivatives for use as kinase inhibitors. WO 2012143248, **2012**.

[20] Brasca, M.G.; Nesi, M.; Avanzi, N.; Ballinari, D.; Bandiera, T.; Bertrand, J.; Bindi, S.; Canevari, G.; Carenzi, D.; Casero, D.; Ceriani, L.; Ciomei, M.; Cirla, A.; Colombo, M.; Cribioli, S.; Cristiani, C.; Della Vedova, F.; Fachin, G.; Fasolini, M.; Felder, E.R.; Galvani, A.; Isacchi, A.; Mirizzi, D.; Motto, I.; Panzeri, A.; Pesenti, E.; Vianello, P.; Gnocchi, P.; Donati, D. Pyrrole-3-carboxamides as potent and selective JAK2 inhibitors. *Bioorg. Med. Chem.*, **2014**, *22*, 4998-5012.

[21] Merck Sharp & Dohme Corp. Cycloalkylnitrile pyrazole carboxamides as Janus kinase inhibitors. WO 2013040863, **2013**.

[22] Merck Sharp & Dohme Corp. Pyrazole carboxamides as Janus kinase inhibitors. WO 2013041042, **2013**.

[23] Merck Sharp & Dohme Corp. Cyanomethylpyrazole carboxamides as Janus kinase inhibitors. WO 2013043962, **2013**.

[24] Merck Sharp & Dohme Corp. Acyclic cyanoethylpyrazoles as Janus kinase inhibitors. WO 2013043964, **2013**.

[25] Incyte Corporation. Preparation of bipyrazole derivatives as JAK kinase inhibitors. WO 2014186706, **2014**.

[26] Liang, X; Huang, Y; Zang, J; Gao, Q; Wang, B; Xu, W; Zhang, Y. Design, synthesis and preliminary biological evaluation of 4-aminopyrazole derivatives as novel and potent JAKs inhibitors. *Bioorg. Med. Chem.*, **2016**, *24*, 2660-2672.

[27] Liang, X; Zang, J; Zhu, M; Gao, Q; Wang, B; Xu, W; Zhang, Y. Design, synthesis, and antitumor evaluation of 4-amino-(1*H*)-pyrazole derivatives as JAKs inhibitors. *ACS Med. Chem. Lett.*, **2016**, *7*, 950-955.

[28] Sang, Y-L.; Duan, Y-T.; Qiu, H-Y.; Wang, P-F.; Makawana, J.A.; Wang, Z-C.; Zhu, H-L.; He, Z-X. Design, synthesis, biological evaluation and molecular docking of novel metronidazole derivatives as selective and potent JAK3 inhibitors. *RSC Adv.*, **2014**, *4*, 16694-16704.

[29] Exelixis Inc. 4-Aryl-2-amino-pyrimidines or 4-aryl-2-aminoalkyl-pyrimidines as JAK-2 modulators and methods of use. WO 2007089768, **2007**.

[30] Targegen Inc. Bi-aryl meta-pyrimidine inhibitors of kinases. WO 2007053452, **2007**.

[31] Cytopia RES Pty Ltd. Phenyl amino pyrimidine compounds and uses thereof. WO 2008109943, **2008**.

[32] YM Biosciences Australia Pty Ltd. Preparation of phenyl amino pyrimidine bicyclic compounds useful as protein kinase inhibitors. WO 2014000032, **2014**.

[33] Calitor Sciences LLC, Sunshine Lake Pharma Co., Ltd. Substituted heteroaryl compounds and methods of use. WO 2015094803, **2015**.

[34] Dana-Faber Cancer Institute, Inc. Janus kinase inhibitors and uses thereof. WO 2015164614, **2015**.

[35] Joshi, M.; Rizvi, S.M.; Belani, C.P. Afatinib for the treatment of metastatic non-small cell lung cancer. *Cancer Manag. Res.*, **2015**, *7*, 75-82.

[36] de Claro, R.A.; McGinn, K.M.; Verdun, N.; Lee, S.L.; Chiu, H.J.; Saber, H.; Brower, M.E.; Chang, C.J.; Pfuma, E.; Habtemariam, B.; Bullock, J.; Wang, Y.; Nie, L.; Chen, X.H.; Lu, D.R.; Al-Hakim, A.; Kane, R.C.; Kaminskas, E.; Justice, R.; Farrell, A.T.; Pazdur, R. FDA approval: Ibrutinib for patients with previously treated mantle cell lymphoma and previously treated chronic lymphocytic leukemia. *Clin. Cancer Res.*, **2015**, *21*, 3586-3590.

[37] Tan, L.; Akahane, K.; McNally, R.; Reyskens, K.M.; Ficarro, S.B.; Liu, S.; Herter-Sprie, G.S.; Koyama, S.; Pattison, M.J.; Labella, K.; Johannessen, L.; Akbay, E.A.; Wong, K.K.; Frank, D.A.; Marto, J.A.; Look, T.A.; Arthur, J.S.; Eck, M.J.; Gray, N.S. Development of selective covalent Janus kinase 3 inhibitors. *J. Med. Chem.*, **2015**, *58*, 6589-6606.

[38] Almirall, S.A. Pyrazolopyrimidin-2-yl derivatives as JAK inhibitors. WO 2015086693, **2015**.

[39] Almirall, S.A. Imidazolopyrimidin-2-yl derivatives as JAK inhibitors. WO 2015091531, **2015**.

[40] Almirall, S.A. Preparation of 2-(pyrazolopyridin-3-yl) pyrimidine derivatives as JAK inhibitors. WO 2016198663, **2016**.

[41] Portola Pharmaceuticals, Inc. Pyridazine compounds as JAK inhibitors. WO 2015123453, **2015**.

[42] Vertex Pharmaceuticals Inc. Azaindoles useful as inhibitors of Janus kinases. WO 2007084557, **2007**.

[43] Astellas Pharma Inc. Heterocyclic Janus kinase 3 inhibitors. WO 2007077949, **2007**.

[44] Farmer, L.J.; Ledeboer, M.W.; Hoock, T.; Arnost, M.J.; Bethiel, R.S.; Bennani, Y.L.; Black, J.J.; Brummel, C.L.; Chakilam, A.; Dorsch, W.A.; Fan, B.; Cochran, J.E.; Halas, S.; Harrington, E.M.; Hogan, J.K.; Howe, D.; Huang, H.; Jacobs, D.H.; Laitinen, L.M.; Liao, S.; Mahajan, S.; Marone, V.; Martinez-Botella, G.; McCarthy, P.; Messersmith, D.; Namchuk, M.; Oh, L.; Penney, M.S.; Pierce, A.C.; Raybuck, S.A.; Rugg, A.; Salituro, F.G.; Saxena, K.; Shannon, D.; Shlyakter, D.; Swenson, L.; Tian, S.K.; Town, C.; Wang, J.; Wang, T.; Wannamaker, M.W.; Winquist, R.J.; Zuccola, H.J. Discovery of VX-509 (Decernotinib): a potent and selective Janus kinase 3 inhibitor for the treatment of autoimmune diseases. *J. Med. Chem.*, **2015**, *58*, 7195-7216.

[45] Iwata, S.; Tanaka, Y. Progress in understanding the safety and efficacy of Janus kinase inhibitors for treatment of rheumatoid arthritis. *Expert Rev. Clin. Immunol.*, **2016**, *12*, 1047-1057.

[46] Merck Sharp & Dohme Corp. Azaindoles as Janus kinase inhibitors. WO 2013052355, **2013**.

[47] Nakajima, Y.; Inoue, T.; Nakai, K.; Mukoyoshi, K.; Hamaguchi, H.; Hatanaka, K.; Sasaki, H.; Tanaka, A.; Takahashi, F.; Kunikawa, S.; Usuda, H.; Moritomo, A.; Higashi, Y.; Inami, M.; Shirakami, S. Synthesis and evaluation of novel 1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxamide derivatives as potent and orally efficacious immunomodulators targeting JAK3. *Bioorg. Med. Chem.*, **2015**, *23*, 4871-4883.

[48] Astellas Pharma Inc. Condensed pyridine compounds. WO 2008084861, **2008**.

[49] Novartis Tiergesundheit AG. Preparation of heterocyclyl-substituted aminosulfonylmethyl cyclohexanes as Janus kinase inhibitors. WO 2015144773, **2015**.

[50] Incyte Corporation. Preparation of piperidin-4-yl azetidine derivatives as JAK1 inhibitors. WO 2011112662, **2011**.

[51] Clinicaltrials.gov. 20 Studies found for: INCB039110. Available at: https://clinicaltrials.gov/ct2/results?term=INCB039110&Search=Search [Accessed: November 24, **2017**].

[52] Bissonnette, R.; Luchi, M.; Fidelus-Gort, R.; Jackson, S.; Zhang, H.; Flores, R.; Newton, R.; Scherle, P.; Yeleswaram, S.; Chen, X.; Menter, A. A randomized, double-blind, placebo-controlled, dose-escalation study of the safety and efficacy of INCB039110, an oral Janus kinase 1 inhibitor, in patients with stable, chronic plaque psoriasis. *J. Dermatolog. Treat.*, **2016**, *27*, 332-338.

[53] Mascarenhas, J.O.; Talpaz, M.; Gupta, V.; Foltz, L.M.; Savona, M.R.; Paquette, R.; Turner, A.R.; Coughlin, P.; Winton, E.; Burn, T.C.; O'Neill, P.; Clark, J.; Hunter, D.; Assad, A.; Hoffman, R.; Verstovsek, S. Primary analysis of a phase II open-label trial of INCB039110, a selective JAK1 inhibitor, in patients with myelofibrosis. *Haematologica*, **2017**, *102*, 327-335.

[54] Incyte Corporation. Cyclohexyl azetidine derivatives as JAK inhibitors. WO 2013026025, **2013**.

[55] Incyte Corporation. Piperidinylcylobutyl substituted pyrrolopyridine and pyrrolopyrimidine derivatives as JAK inhibitors. WO 2013173720, **2013**.

[56] Merck Sharp & Dohme Corp. Preparation of pyrrolopyrimidines as Janus kinase inhibitors. WO 2013085802, **2013**.

[57] Elwood, F.; Witter, D.J.; Piesvaux, J.; Kraybill, B.; Bays, N.; Alpert, C.; Goldenblatt, P.; Qu, Y.; Ivanovska, I.; Lee, H.H.; Chiu, C.S.; Tang, H.; Scott, M.E.; Deshmukh, S.V.; Zielstorff, M.; Byford, A.; Chakravarthy, K.; Dorosh, L.; Rivkin, A.; Klappenbach, J.; Pan, B.S.; Kariv, I.; Dinsmore, C.; Slipetz, D.; Dandliker, P.J. Evaluation of JAK3 biology in autoimmune disease using a highly selective, irreversible JAK3 inhibitor. *J. Pharmacol. Exp. Ther.*, **2017**, *361*, 229-244.

[58] Pfizer Inc. Pyrrolo[2,3-*d*]pyrimidine derivative as inhibitors of Janus-related kinases (JAK) and their preparation. WO 2014128591, **2014**.

[59] Pfizer Inc. Pyrrolo[2,3-*d*]pyrimidinyl, pyrrolo[2,3-*b*] pyrazinyl and pyrrolo[2,3-*d*]pyridinyl acrylamides as JAK inhibitor and their preparation. WO 2015083028, **2015**.

[60] Pfizer Inc. Pyrrolo[2,3-*d*]pyrimidinyl, pyrrolo[2,3-*b*] pyrazinyl, pyrrolo[2,3-*b*]pyridinyl acrylamides and epoxides thereof. WO 2016178110, **2016**.

[61] Thorarensen, A.; Dowty, M.E.; Banker, M.E.; Juba, B.; Jussif, J.; Lin, T.; Vincent, F.; Czerwinski, R.M.; Casimiro-Garcia, A.; Unwalla, R.; Trujillo, J.I.; Liang, S.; Balbo, P.; Che, Y.; Gilbert, A.M.; Brown, M.F.; Hayward, M.; Montgomery, J.; Leung, L.; Yang, X.; Soucy, S.; Hegen, M.; Coe, J.; Langille, J.; Vajdos, F.; Chrencik, J.; Telliez, J.B. Design of a Janus kinase 3 (JAK3) specific inhibitor 1-((2*S*,5*R*)-5-((7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)-2-methylpiperidin-1-yl)prop-2-en-1-one (PF-06651600) allowing for the interrogation of JAK3 signaling in humans. *J. Med. Chem.*, **2017**, *60*, 1971-1993.

[62] Telliez, J.B.; Dowty, M.E.; Wang, L.; Jussif, J.; Lin, T.; Li, L.; Moy, E.; Balbo, P.; Li, W.; Zhao, Y.; Crouse, K.; Dickinson, C.; Symanowicz, P.; Hegen, M.; Banker, M.E.; Vincent, F.; Unwalla, R.; Liang, S.; Gilbert, A.M.; Brown, M.F.; Hayward, M.; Montgomery, J.; Yang, X.; Bauman, J.; Trujillo, J.I.; Casimiro-Garcia, A.; Vajdos, F.F.; Leung, L.; Geoghegan, K.F.; Quazi, A.; Xuan, D.; Jones, L.; Hett, E.; Wright, K.; Clark, J.D.; Thorarensen, A. Discovery of a JAK3-selective inhibitor: functional differentiation of JAK3-selective inhibition over pan-JAK or JAK1-selective inhibition. *ACS Chem. Biol.*, **2016**, *11*, 3442-3451.

[63] Clinicaltrials.gov. 6 Studies found for: PF-06651600. Available at: https://clinicaltrials.gov/ct2/results?cond=&term=PF-06651600&cntry1=&state1=&recrs= [Accessed: November 24, **2017**].

[64] Wang, T.; Liu, X.; Hao, M.; Qiao, J.; Ju, C.; Xue, L.; Zhang, C. Design, synthesis and evaluation of pyrrolo[2,3-*d*]pyrimidine-phenylamide hybrids as potent Janus kinase 2 inhibitors. *Bioorg. Med. Chem. Lett.*, **2016**, *26*, 2936-2941.

[65] Lee, S.M.; Yoon, K.B.; Lee, H.J.; Kim, J.; Chung, Y.K.; Cho, W.J.; Mukai, C.; Choi, S.; Kang, K.W.; Han, S.Y.; Ko, H.; Kim, Y.C. The discovery of 2,5-isomers of triazole-pyrrolopyrimidine as selective Janus kinase 2 (JAK2) inhibitors *versus* JAK1 and JAK3. *Bioorg. Med. Chem.*, **2016**, *24*, 5036-5046.

[66] Soth, M.; Hermann, J.C.; Yee, C.; Alam, M.; Barnett, J.W.; Berry, P.; Browner, M.F.; Frank, K.; Frauchiger, S.; Harris, S.; He, Y.; Hekmat-Nejad, M.; Hendricks, T.; Henningsen, R.; Hilgenkamp, R.; Ho, H.; Hoffman, A.; Hsu, P.Y.; Hu, D.Q.; Itano, A.; Jaime-Figueroa, S.; Jahangir, A.; Jin, S.; Kuglstatter, A.; Kutach, A.K.; Liao, C.; Lynch, S.; Menke, J.; Niu, L.; Patel, V.; Railkar, A.;Roy, D.; Shao, A.; Shaw, D.; Steiner, S.; Sun, Y.; Tan, S.L.; Wang, S.; Vu, M.D. 3-Amido pyrrolopyrazine JAK kinase inhibitors: development of a JAK3 *vs* JAK1 selective inhibitor and evaluation in cellular and *in vivo* models. *J. Med. Chem.*, **2013**, *56*, 345-356.

[67] Jaime-Figueroa, S.; De Vicente, J.; Hermann, J.; Jahangir, A.; Jin, S.; Kuglstatter, A.; Lynch, S.M.; Menke, J.; Niu, L.; Patel, V.; Shao, A.; Soth, M.; Vu, M.D.; Yee, C. Discovery of a series of novel 5*H*-pyrrolo[2,3-*b*]pyrazine-2-phenyl ethers, as potent JAK3 kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 2522-2526.

[68] de Vicente, J.; Lemoine, R.; Bartlett, M.; Hermann, J.C.; Hekmat-Nejad, M.; Henningsen, R.; Jin, S.; Kuglstatter, A.; Li, H.; Lovey, A.J.; Menke, J.; Niu, L.; Patel, V.; Petersen, A.; Setti, L.; Shao, A.; Tivitmahaisoon, P.; Vu, M.D.; Soth, M. Scaffold hopping towards potent and selective JAK3 inhibitors: discovery of novel C-5 substituted pyrrolopyrazines. *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 4969-4975.

[69] Duan, J.J.; Lu, Z.; Jiang, B.; Yang, B.V.; Doweyko, L.M.; Nirschl, D.S.; Haque, L.E.; Lin, S.; Brown, G.; Hynes, J. Jr; Tokarski, J.S.; Sack, J.S.; Khan, J.; Lippy, J.S.; Zhang, R.F.; Pitt, S.; Shen, G.; Pitts, W.J.; Carter, P.H.; Barrish, J.C.; Nadler, S.G.; Salter-Cid, L.M.; McKinnon, M.; Fura, A.; Schieven, G.L.; Wrobleski, S.T. Discovery of pyrrolo[1,2-*b*]pyridazine-3-carboxamides as Janus kinase (JAK) inhibitors. *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 5721-5726.

[70] *Bristol-Myers Squibb* Co. Pyrrolopyridazine Jak3 inhibitors and their use for the treatment of inflammatory and autoimmune diseases. WO 2012125886, **2012**.

[71] Pfizer Ltd. Indazoles. WO 2013014567, **2013**.

[72] Jones, P.; Storer, R.I.; Sabnis, Y.A.; Wakenhut, F.M.; Whitlock, G.A.; England, K.S.; Mukaiyama, T.; Dehnhardt, C.M.; Coe, J.W.; Kortum, S.W.; Chrencik, J.E.; Brown, D.G.; Jones, R.M.; Murphy, J.R.; Yeoh, T.; Morgan, P.; Kilty, I. Design and synthesis of a pan-Janus kinase inhibitor clinical candidate (PF-06263276) suitable for inhaled and topical delivery for the treatment of inflammatory diseases of the lungs and skin. *J. Med. Chem.*, **2017**, *60*, 767-786.

[73] Topivert Pharma Ltd. Preparation of 4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine and 1,4,5,6,7,8-hexahydro-1H-imidazo[4,5-*d*]diazepine derivatives as Janus kinase inhibitors. WO 2017077283, **2017**.

[74] Topivert Pharma Ltd. Preparation of 4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine and 1,4,5,6,7,8-hexahydroimidazo [4,5-*d*]azepine derivatives as Janus kinase inhibitors. WO 2017077288, **2017**.

[75] Ritzén, A.; Sørensen, M.D.; Dack, K.N.; Greve, D.R.; Jerre, A.; Carnerup, M.A.; Rytved, K.A.; Bagger-Bahnsen, J. Fragment-based discovery of 6-arylindazole JAK inhibitors. *ACS Med. Chem. Lett.*, **2016**, *7*, 641-646.

[76] Cellzome Ltd. Pyrazolo[4,3-*c*]pyridine derivatives as Jak inhibitors. WO 2013017479, **2013**.

[77] Cellzome Ltd. Pyrazolo[4,3-*c*]pyridine derivatives as Jak inhibitors. WO 2013017480, **2013**.

[78] Merck Sharp & Dohme Corp. Cycloalkyl nitrile pyrazolo pyridones as Janus kinase inhibitors. WO 2014146246, **2014**.

[79] Merck Sharp & Dohme Corp. Geminally substituted cyanoethylpyrazolo pyridones as Janus kinase inhibitors. WO 2014146249, **2014**.

[80] Merck Sharp & Dohme Corp. N-(2-cyano heterocyclyl)pyrazolo pyridones as Janus kinase inhibitors. WO 2014146492, **2014**.

[81] Merck Sharp & Dohme Corp. Acyclic cyanoethylpypyrazolo pyridones as Janus kinase inhibitors. WO 2014146493, **2014**.

[82] Merck Sharp & Dohme Corp. Ethyl N-Boc piperidinyl pyrazolo pyridones as Janus kinase inhibitors. 2016 WO 2016064935, **2016**.

[83] Merck Sharp & Dohme Corp. Ethyl N-Boc piperidinyl pyrazolo pyridones as Janus kinase inhibitors. WO 2016061751, **2016**.

[84] Pfizer Inc. Pyrazolopyridines and pyrazolopyrimidines as JAK inhibitors and their preparation. WO 2015173683, **2015**.

[85] Calitor Sciences LLC, Sunshine Lake Pharma Co., Ltd. Preparation of substituted pyrazolo[1,5-*a*]pyrimidine-3-carboxamide derivatives as JAK kinase inhibitors. WO 2015073267, **2015**.

[86] Celon Pharma S.A. Pyrazolo[1,5-*a*]pyrimidine derivatives as kinase JAK-2 inhibitors. WO 2015118434, **2015**.

[87] F. Hoffmann-La Roche AG. 5-Chloro-2-difluoromethoxyphenyl pyrazolopyrimidine compounds which are JAK inhibitors. WO 2015177326, **2015**.

[88] Array Biopharma Inc. 4,6-Disubstituted-pyrazolo[1,5-*a*]pyrazines as Janus kinase inhibitors and their preparation. WO 2016090285, **2016**.

[89] Meyer, S.C.; Keller, M.D.; Chiu, S.; Koppikar, P.; Guryanova, O.A.; Rapaport, F.; Xu, K.; Manova, K.; Pankov, D.; O'Reilly, R.J.; Kleppe, M.; McKenney, A.S.; Shih, A.H.; Shank, K.; Ahn, J.; Papalexi, E.; Spitzer, B.; Socci, N.; Viale, A.; Mandon, E.; Ebel, N.; Andraos, R.; Rubert, J.; Dammassa, E.; Romanet, V.; Dölemeyer, A.; Zender, M.; Heinlein, M.; Rampal, R.; Weinberg, R.S.; Hoffman, R.; Sellers, W.R.; Hofmann, F.; Murakami, M.; Baffert, F.; Gaul, C.; Radimerski, T.; Levine, R.L. CHZ868, a type II JAK2 inhibitor, reverses type I JAK inhibitor persistence and demonstrates efficacy in myeloproliferative neoplasms. *Cancer Cell.*, **2015**, *28*, 15-28.

[90] Wu, S.C.; Li, L.S.; Kopp, N.; Montero, J.; Chapuy, B.; Yoda, A.; Christie, A.L.; Liu, H.; Christodoulou, A.; van Bodegom, D.; van der Zwet, J.; Layer, J.V.; Tivey, T.; Lane, A.A.; Ryan, J.A.; Ng, S.Y.; DeAngelo, D.J.; Stone, R.M.; Steensma, D.; Wadleigh, M.; Harris, M.; Mandon, E.; Ebel, N.; Andraos, R.; Romanet, V.; Dölemeyer, A.; Sterker, D.; Zender, M.; Rodig, S.J.; Murakami, M.; Hofmann, F.; Kuo, F.; Eck, M.J.; Silverman, L.B.; Sallan, S.E.; Letai, A.; Baffert, F.; Vangrevelinghe, E.; Radimerski, T.; Gaul, C.; Weinstock, D.M. Activity of the type II JAK2 inhibitor CHZ868 in B cell acute lymphoblastic leukemia. *Cancer Cell.*, **2015**, *28*, 29-41.

[91] Kim, M.K.; Shin, H.; Park, K.S.; Kim, H.; Park, J.; Kim, K.; Nam, J.; Choo, H.; Chong, Y. Benzimidazole derivatives as potent JAK1-selective inhibitors. *J. Med. Chem.*, **2015**, *58*, 7596-7602.

[92] Simov, V.; Deshmuckh, S. V.; Dinsmore C.J.; Elwood, F.; Fernandez R.B.; Garcia, Y.; Gibeau, C.; Gunaydin, H.; Jung, J.; Katz, J.D.; Kraybill, B.; Lapointe, B.; Patel, S.B.; Siu, T.; Su, H.; Young, J.R. Structure-based design and development of (benz)imidazole pyridones as JAK1-selective kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2016**, *26*, 1803-1808.

[93] JN Therapeutics. Preparation of substituted imidazo[1,2-*a*]pyridin-2-ylamine compounds for treating, preventing, or ameliorating one or more symptoms of a Janus kinase-mediated disease. WO 2016119700, **2016**.

[94] Vasbinder, M.M.; Alimzhanov, M.; Augustin, M.; Bebernitz, G.; Bell, K.; Chuaqui, C.; Deegan, T.; Ferguson, A.D.; Goodwin, K.; Huszar, D.; Kawatkar, A.; Kawatkar, S.; Read, J.; Shi, J.; Steinbacher, S.; Steuber, H.; Su, Q.; Toader, D.; Wang, H.; Woessner, R.; Wu, A.; Ye, M.; Zinda, M. Identification of azabenzimidazoles as potent JAK1 selective inhibitors. *Bioorg. Med. Chem. Lett.*, **2016**, *26*, 60-67.

[95] Galapagos NV. Imidazo[4,5-*c*]pyridine derivatives as JAK kinase inhibitors useful for the treatment of degenerative

and inflammatory diseases and their preparation. WO 2013117645, **2013**.

[96] Galapagos NV. Preparation of *N*-[4-ethyl-6-[1-(methylsulfonyl)-3-azetidinyl]-3-pyridinyl]-N,1-dimethyl-1*H*-imidazo[4,5-*c*]pyridin-6-amine as JAK inhibitor useful for the treatment of degenerative and inflammatory diseases. WO 2014111380, **2014**.

[97] Galapagos NV. Imidazo[4,5-*c*]pyridine derivatives useful for the treatment of degenerative and inflammatory diseases. WO 2013117645, **2013**.

[98] Galapagos NV. Novel compound useful for the treatment of degenerative and inflammatory diseases. WO 2013117646, **2013**.

[99] Galapagos NV. Imidazo[4,5-*c*]pyridine derivatives as JAK kinase inhibitors useful for the treatment of degenerative and inflammatory diseases and their preparation WO 2013117649, **2013**.

[100] Galapagos NV. Preparation of pyridoimidazoles and pharmaceutical compositions thereof for the treatment of inflammatory disorders. WO 2017012647, **2017**.

[101] Eli-Lilly & Co. Amino pyrazole compounds. WO 2010074947, **2010**.

[102] Ma, L.; Clayton, J.R.; Walgren, R.A.; Zhao, B.; Evans, R.J.; Smith, M.C.; Heinz-Taheny, K.M.; Kreklau, E.L.; Bloem, L.; Pitou, C.; Shen, W.; Strelow, J.M.; Halstead, C.; Rempala, M.E.; Parthasarathy, S.; Gillig, J.R.; Heinz, L.J.; Pei, H.; Wang, Y.; Stancato, L.F.; Dowless, M.S.; Iversen, P.W.; Burkholder, T.P. Discovery and characterization of LY2784544, a small-molecule tyrosine kinase inhibitor of JAK2V617F. *Blood Cancer J.*, **2013**, *3*, e109.

[103] Clinicaltrials.gov. 4 Studies found for: LY2784544. https://clinicaltrials.gov/ct2/results?cond=&term=LY2784544&cntry1=&state1=&Search=Search (Accessed November 24, **2017**).

[104] Celon Pharma S.A. Preparation of imidazo[1,2-*b*]pyridazin-6-amine derivatives as JAK2 inhibitors. WO 2014020531, **2014**.

[105] Array Biopharma Inc. Preparation of imidazopyrimidine derivatives for use as Jak kinase inhibitors. WO 2013055645, **2013**.

[106] Ligand Pharmaceuticals Inc. Janus kinase inhibitor compounds and methods. WO 2013025628, **2013**.

[107] Galapagos NV. Novel compounds useful for the treatment of degenerative and inflammatory diseases. WO 2010010190, **2010**.

[108] Menet, C.J.; Fletcher, S.R.; Van Lommen, G.; Geney, R.; Blanc, J.; Smits, K.; Jouannigot, N.; Deprez, P.; van der Aar, E.M.; Clement-Lacroix, P.; Lepescheux, L.; Galien, R.; Vayssiere, B.; Nelles, L.; Christophe, T.; Brys, R.; Uhring, M.; Ciesielski, F.; Van Rompaey, L. Triazolopyridines as selective JAK1 inhibitors: from hit identification to GLPG0634. *J. Med. Chem.*, **2014**, *57*, 9323-9342.

[109] Danese, S.; Fiorino, G.; Peyrin-Biroulet, L. Filgotinib in Crohn's disease: JAK is back. *Gastroenterology*, **2017**, *153*, 603-605.

[110] Taylor, P.C.; Abdul Azeez, M.; Kiriakidis, S. Filgotinib for the treatment of rheumatoid arthritis. *Expert Opin. Investig. Drugs*, **2017**, *26*, 1181-1187.

[111] Clinicaltrials.gov. 20 Studies found for: filgotinib. https://clinicaltrials.gov/ct2/results?cond=&term=filgotinib&cntry1=&state1=&recrs (Accessed November 24, **2017**).

[112] Galapagos NV. Aminotriazolopyridine for use in the treatment of inflammation, and pharmaceutical compositions thereof. WO 2013189771, **2013**.

[113] F. Hoffmann-La Roche AG. Preparation of triazolopyridine compounds as Janus kinase inhibitors and methods of use thereof. WO 2016139212, **2016**.

[114] Labadie, S.; Barrett, K.; Blair, W.S.; Chang, C.; Deshmukh, G.; Eigenbrot, C.; Gibbons, P.; Johnson, A.; Kenny, J.R.; Kohli, P.B.; Liimatta, M.; Lupardus, P.J.; Shia, S.; Steffek, M.; Ubhayakar, S.; van Abbema, A.; Zak, M. Design and evaluation of novel 8-oxo-pyridopyrimidine Jak1/2 inhibitors. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 5923-5930.

[115] Theravance Biopharma R&D IP, LLC. Naphthyridine compounds as Jak kinase inhibitors. WO 2016191524, **2016**.

[116] Jiangsu Simcere Pharmaceutical Co., Ltd. Pyrimidine compounds and use thereof. WO 2014075318, **2014**.

[117] Wang, Y.; Huang, W.; Xin, M.; Chen, P.; Gui, L.; Zhao, X.; Tang, F.; Wang, J.; Liu, F. Identification of 4-(2-furanyl)pyrimidin-2-amines as Janus kinase 2 inhibitors. *Bioorg. Med. Chem.*, **2017**, *25*, 75-83.

[118] Gehringer, M.; Pfaffenrot, E.; Bauer, S.; Laufer, S.A. Design and synthesis of tricyclic JAK3 inhibitors with picomolar affinities as novel molecular probes. *ChemMedChem*, **2014**, *9*, 277-281.

[119] Astellas Pharma Inc. Fused pyrrolopyridine derivative. WO 2010119875, **2010**.

[120] Nakamura, K.; Inami, M.; Morio, H.; Okuma, K.; Ito, M.; Noto, T.; Shirakami, S.; Hirose, J.; Morokata, T. AS2553627, a novel JAK inhibitor, prevents chronic rejection in rat cardiac allografts. *Eur. J. Pharmacol.*, **2017**, *796*, 69-75.

[121] *Bristol-Myers Squibb* Co. JAK2 inhibitors and their use for the treatment of myeloproliferative diseases and cancer. WO 2011028864, **2011**.

[122] Wan, H.; Schroeder, G.M.; Hart, A.C.; Inghrim, J.; Grebinski, J.; Tokarski, J.S.; Lorenzi, M.V.; You, D.; Mcdevitt, T.; Penhallow, B.; Vuppugalla, R.; Zhang, Y.; Gu, X.; Iyer, R.; Lombardo, L.J.; Trainor, G.L.; Ruepp, S.; Lippy, J.; Blat, Y.; Sack, J.S.; Khan, J.A.; Stefanski, K.; Sleczka, B.; Mathur, A.; Sun, J.H.; Wong, M.K.; Wu, D.R.; Li, P.; Gupta, A.; Arunachalam, P.N.; Pragalathan, B.; Narayanan, S.; Nanjundaswamy, K.C.; Kuppusamy, P.; Purandare, A.V. Discovery of a highly selective JAK2 Inhibitor, BMS-911543, for the treatment of myeloproliferative neoplasms. *ACS Med. Chem. Lett.*, **2015**, *6*, 850-855.

[123] Mace, T.A.; Shakya, R.; Elnaggar, O.; Wilson, K.; Komar, H.M.; Yang, J.; Pitarresi, J.R.; Young, G.S.; Ostrowski, M.C.; Ludwig, T.; Bekaii-Saab, T.; Bloomston, M.; Lesinski, G.B. Single agent BMS-911543 Jak2 inhibitor has distinct inhibitory effects on STAT5 signaling in genetically engineered mice with pancreatic cancer. *Oncotarget*, **2015**, *6*, 44509-44522.

[124] Pomicter, A.D.; Eiring, A.M.; Senina, A.V.; Zabriskie, M.S.; Marvin, J.E.; Prchal, J.T.; O'Hare, T.; Deininger, M.W. Limited efficacy of BMS-911543 in a murine model of Janus kinase 2 V617F myeloproliferative neoplasm. *Exp. Hematol.*, **2015**, *43*, 537-545.

[125] Hart, A.C.; Schroeder, G.M.; Wan, H.; Grebinski, J.; Inghrim, J.; Kempson, J.; Guo, J.; Pitts, W.J.; Tokarski, J.S.; Sack, J.S.; Khan, J.A.; Lippy, J.; Lorenzi, M.V.; You, D.; McDevitt, T.; Vuppugalla, R.; Zhang, Y.; Lombardo, L.J.; Trainor, G.L.; Purandare, A.V. Structure-based design of selective Janus kinase 2 imidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridine inhibitors. *ACS Med. Chem. Lett.*, **2015**, *6*, 845-849.

[126] Gehringer, M.; Forster, M.; Pfaffenrot, E.; Bauer, S.M.; Laufer, S.A. Novel hinge-binding motifs for Janus kinase 3 inhibitors: a comprehensive structure-activity relationship study on tofacitinib bioisosteres. *ChemMedChem*, **2014**, *9*, 2516-2527.

[127] F. Hoffmann-La Roche AG. Fused tricyclic compounds for use as inhibitors of Janus kinases and their preparation. WO 2013007765, **2013**.

[128] Goedken, E.R.; Argiriadi, M.A.; Banach, D.L.; Fiamengo, B.A.; Foley, S.E.; Frank, K.E.; George, J.S.; Harris, C.M.; Hobson, A.D.; Ihle, D.C.; Marcotte, D.; Merta, P.J.; Michalak, M.E.; Murdock, S.E.; Tomlinson, M.J.; Voss, J.W. Tricyclic covalent inhibitors selectively target Jak3 through an active site thiol. *J. Biol. Chem.*, **2015**, *290*, 4573-4589.

[129] Yamagishi, H.; Shirakami, S.; Nakajima, Y.; Tanaka, A.; Takahashi, F.; Hamaguchi, H.; Hatanaka, K.; Moritomo, A.; Inami, M.; Higashi, Y.; Inoue, T. Discovery of 3,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-2(1*H*)-one derivatives as novel JAK inhibitors. *Bioorg. Med. Chem.*, **2015**, *23*, 4846-4859.

[130] Forster, M.; Chaikuad, A.; Bauer, S.M.; Holstein, J.; Robers, M.B.; Corona, C.R.; Gehringer, M.; Pfaffenrot, E.; Ghoreschi, K.; Knapp, S.; Laufer, S.A. Sselective Jak3 inhibitors with a covalent reversible binding mode targeting a new induced fit binding pocket. *Cell. Chem. Biol.*, **2016**, *23*, 1335-1340.

[131] Hurley, C.A.; Blair, W.S.; Bull, R.J.; Chang, C.; Crackett, P.H.; Deshmukh, G.; Dyke, H.J.; Fong, R.; Ghilardi, N.; Gibbons, P.; Hewitt, P.R.; Johnson, A.; Johnson, T.; Kenny, J.R.; Kohli, P.B.; Kulagowski, J.J.; Liimatta, M.; Lupardus, P.J.; Maxey, R.J.; Mendonca, R.; Narukulla, R.; Pulk, R.; Ubhayakar, S.; van Abbema, A.; Ward, S.I.; Waszkowycz, B.; Zak, M. Novel triazolo-pyrrolopyridines as inhibitors of Janus kinase 1. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 3592-3598.

[132] Friedman, M.; Frank, K.E.; Aguirre, A.; Argiriadi, M.A.; Davis, H.; Edmunds, J.J.; George, D.M.; George, J.S.; Goedken, E.; Fiamengo, B.; Hyland, D.; Li, B.; Murtaza, A.; Morytko, M.; Somal, G.; Stewart, K.; Tarcsa, E.; Van Epps, S.; Voss, J.; Wang, L.; Woller, K.; Wishart, N. Structure activity optimization of 6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazines as Jak1 kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2015**, *25*, 4399-4404.

[133] F. Hoffmann-La Roche AG. Tricyclic heterocyclic compounds, compositions and methods of use thereof as Jak inhibitors. WO 2013007768, **2013**.

[134] Siu, T.; Kumarasinghe, S.E.; Altman, M.D.; Katcher, M.; Northrup, A.; White, C.; Rosenstein, C.; Mathur, A.; Xu, L.; Chan, G.; Bachman, E.; Bouthillette, M.; Dinsmore, C.J.; Marshall, C.G.; Young, J.R. The discovery of reverse tricyclic pyridone JAK2 inhibitors. Part 2: lead optimization. *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 1466-1471.

[135] Zimmermann, K.; Sang, X.; Mastalerz, H.A.; Johnson, W.L.; Zhang, G.; Liu, Q.; Batt, D.; Lombardo, L.J.; Vyas, D.; Trainor, G.L.; Tokarski, J.S.; Lorenzi, M.V.; You, D.; Gottardis, M.M.; Lippy, J.; Khan, J.; Sack, J.S.; Purandare, A.V. 9*H*-Carbazole-1-carboxamides as potent and selective JAK2 inhibitors. *Bioorg. Med. Chem. Lett.*, **2015**, *25*, 2809-2812.

[136] *Bristol-Myers Squibb* Co. Carbazole and carboline kinase inhibitors. WO 2010080474, **2010**.

[137] Gigante, M.; Pontrelli, P.; Herr, W.; Gigante, M.; D'Avenia, M.; Zaza, G.; Cavalcanti, E.; Accetturo, M.; Lucarelli, G.; Carrieri, G.; Battaglia, M.; Storkus, W.J.; Gesualdo, L.; Ranieri, E. miR-29b and miR-198 overexpression in CD8+ T cells of renal cell carcinoma patients down-modulates JAK3 and MCL-1 leading to immune dysfunction. *J. Transl. Med.*, **2016**, *14*, 84.