Anilino-monoindolylmaleimides as potent and selective JAK3 inhibitors

Mark E. McDonnell a, Haiyan Biana, Jay Wrobela,⇑, Garry R. Smitha, Shuguang Liang b, Haiching Ma b, Allen B. Reitz a

a Fox Chase Chemical Diversity Center, Inc., 3805 Old Easton Road, Doylestown, PA 18902, United States
b Reaction Biology, Corp., One Great Valley Parkway, Suite 2, Malvern, PA 19355, United States

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ABSTRACT

We designed a series of anilino-indolylmaleimides based on structural elements from literature JAK3 inhibitors 3 and 4, and our lead 5. These new compounds were tested as inhibitors of JAKs 1, 2 and 3 and TYK2 for therapeutic intervention in rheumatoid arthritis (RA). Our requirements, based on current scientific rationale for optimum efficacy against RA with reduced side effects, was for potent, mixed JAK1 and 3 inhibition, and selectivity over JAK2. Our efforts yielded a potent JAK3 inhibitor 11d and its eutomer 11e. These compounds were highly selective for inhibition of JAK3 over JAK2 and TYK. The compounds displayed only modest JAK1 inhibition.

The four cytoplasmic tyrosine kinases in the family named Janus Kinases (JAK1, JAK2, JAK3 and TYK2) play critical roles in the cytokine receptor binding-triggered signal transduction through Signal Transducers and Activators of Transcription (STATs). Several small molecule JAK inhibitors have now shown success in clinical trials and two have been approved by the FDA for therapeutic use. Tofacitinib 1–4, a potent pan-JAK inhibitor was approved for rheumatoid arthritis (RA) in 2012. Its side effect profile is consistent with its mode of action: upper respiratory infections attributable to its JAK1 inhibitory activity, anemia and neutropenia linked to its JAK2 inhibitory activity and elevated LDL levels possibly caused by its effects on both JAK1 and 2.3,5 Ruxolitinib 2 is a JAK1,2 inhibitor that was approved in 2011 for myelofibrosis and is currently being evaluated in the clinic for RA.5 Other JAK inhibitors are also being investigated in humans for RA including those that are reported to be selective for JAK1 (GLPG0634), JAK2 (CEP33779) and JAK3 (VX-509).3,5 Interestingly these latter three, despite different JAK selectivity preferences, have demonstrated efficacy in RA in Phase II studies.5

We thought we could derive the maximum benefit for a safe and effective RA therapeutic agent through the development of a potent JAK1 and JAK3 mixed inhibitor with selectivity over JAK2 based on the following considerations. JAK2 inhibition would not be desirable due to its potential to induce anemia as seen in the non-selective agents above. JAK3 inhibition is...
preferable since it is found predominantly in hematopoietic cells while JAK1 and 2, and TYK2 are ubiquitously expressed. Although some investigators have argued that JAK3 selectivity may be sufficient for treatment of RA via stimulation through IL-2 pathway, JAK1 and JAK3 both cooperate in signaling through the γc-containing cytokine receptors, but JAK1 is thought to play a dominant role. Therefore inhibition of both JAK1 and 3 may be required for optimum efficacy.

Agents with the mixed JAK profile we desired have not yet been described. Several different investigators reported compounds with varying selectivity for JAK3. 

Compound 3 was reported by Novartis researchers to have particularly high selectivity for JAK3 over JAK1, JAK2, and TYK2. The authors provided an X-ray co-crystal structure of a related analog (4) to explain this high selectivity. Other researchers described JAK1 inhibitors that were selective against JAK2 but JAK3 activity was not reported. Recently, a series of diamino-triazole derivatives were shown to have selectivity for TYK2 and JAK1 over JAK2 and 3. In the last several years JAK2 selective inhibitors have also been disclosed. To date, no analogs with potent JAK1 and 3 inhibitory activities that were selective over JAK2 have been reported.

We recently profiled a number of known kinase inhibitors in our Kinase HotspotSM assay. One compound in particular, PKCβ inhibitor JP539654, showed strong inhibition of JAK3, modest inhibition of JAK1, and excellent selectivity over JAK2 and TYK2 (Table 1). The potent PKCβ inhibitor activity of this compound was confirmed in our assay (data not shown). The profile of this compound was similar to compound 3 in that JAK1 inhibition activity, although considerably weaker than JAK3 inhibition activity, was superior to the inhibition activity of JAK2. According to the X-ray model, the JAK3 selectivity of 3 and 4 is due to an H-bond between the maleimide 2-carbonyl oxygen atom of 4 and D967 of JAK3. A similar H-bond cannot occur between 4 and JAK5 1, 2 or TYK2. This is because these isozymes have a glycine adjacent to this aspartate while JAK3 has an alanine (A966) at this position. The A966 induces a conformation of the adjacent D967 to form a H-bond, via a bridged water molecule, to the maleimide carbonyl oxygen atom of 4.

We reasoned that the JAK3 selectivity of 5 might be due to a bioactive conformation where the distal 5-maleimide carbonyl oxygen atom H-bonds (via a bridged water molecule) to D967 thus orienting the maleimide 4-substituent of 5 (i.e. the indole containing the propyl-imidazole unit) into the region of JAK3 occupied by the 3-phenyl piperidinone substituent of 4. To support this hypothesis, we carried out a small molecule molecular modeling study to show that a low energy conformation of 5 could be superimposed with the X-ray conformation of 4 (Fig. 1) and that their respective side chains have substantial overlap. We envisioned that we could produce novel JAK3 inhibitors by removing the side chain of 5 and appending it to the 4-phenylamino ring as shown by 6. Within this proposed framework we could examine the nature and length of the appending group [X and (CH₂)n] and explore different heterocyclic moieties in an effort to maintain or increase JAK3 inhibitor potency, selectivity over JAK2 and increase potency for JAK1. In fact small molecule modeling revealed that low energy conformations of compounds designed from framework 6, such as 7a, (Fig. 2) could be superimposed with the X-ray conformation of 4 with good overlap of their respective side chains, and this provided impetus for our foray into the synthesis of these analogs.

The synthesis of compounds related to 6 is shown in Schemes 1–3. The 4-anilino-maleimides 7, 8, 9, 10a and 11 were prepared via reaction of 3-hydroxymaleimide 13 with the anilines (14) heated in acetic acid according to established methods (Scheme 1). The starting 4-hydroxy maleimides 13 were commercially available (Ar = 3-indolyl) or prepared from the corresponding 3-substituted acetamide 12 (Ar = phenyl, 1-methyl-3-indolyl) using literature procedures. 4-Bromo-maleimide 15 was commercially available and used to prepare 10b. The starting anilines 14 were prepared by procedures shown in Schemes 2 and 3. Starting nitro-bromides 16 were commercially available. The reaction of amines such as 18, 19, 21 and 22 with 2,4-difluoropyridine is known to add to the pyridine 4-position to ultimately provide, in our case, the 4-amino-2-fluoro-pyridine regiochemistry for 14f–k, o–w. The starting nitro-amines 21 that afforded anilines 14o–u and final compounds 11a–c, g–i were commercially available. To produce enantiomerically pure anilines 14v and w for the production of target compounds 11e and f, we used commercially available S or R-pyrrolidin-3-ol (22 for the S isomer). The key step in the synthesis of 14v and w was reaction of the hydroxyl containing compound 23 (or its enantiomer) with 1-iodo-3-nitrobenene using copper catalyzed conditions described by Buchwald to form the corresponding 3-nitrophenoxypyrene congener. The nitro group of this compound was then reduced to the aniline 14v. The method of preparation of 11e is given in the reference section. The (R)-enantiomer (14w, for 11f) was synthesized in analogous fashion from (R)-pyrrolidinol. The imidazole containing analogs 7a–e are shown in Table 2. Our small molecule modeling suggested that meta and para side chains on the aniline should be able to access conformations that have substantial overlap with the side chains of 3 and 4 when the molecules are superimposed. However side chains from the ortho position should have a steric clash with the 4-maleimide indole moiety thus preventing a conformation similar to the bioactive ones of 3 and 4, or clash with JAK3 residues within the binding site. Indeed the ortho analog 7e had substantially poorer activity against all the JAK isoforms than did the meta and para analogs 7a–d. With respect to the meta versus para positions, the longer side chain (n = 3) of the meta isomer (7a) was superior in potency for JAK3 inhibition to the shorter isomer 7b (n = 2). The opposite was true for the para isomers in that the shorter chain 7c, (n = 2) was superior in potency to the longer side chain 7d.
n = 3). The meta isomer 7a also showed the best potency of this set with respect to JAK1 inhibition. Although none of the compounds exhibited the potency we required, our crude model proved useful and gave us confidence that we could improve potency, at lease with respect to JAK3 inhibition, with additional analog synthesis.

We probed our model further using a side chain with a 2-fluoro-4-aminopyridine terminal unit (Table 3). Once again the ortho isomer (8c) showed poor activity. The para isomers (8a, b) were not substantially better than the para isomer in the imidazole series (7c, d; Table 2). However, the meta isomer (8d) showed a four-fold improvement in potency over its imidazole counterpart 7a (note the chain length atom counts of 7a and 8d are the same, i.e. a four atom spacer between the phenyl and heterocyclic rings). The JAK2 potency also increased although the compound was still over 70 fold selective for JAK3 over JAK2. N-methylation of the indole nitrogen (8e) led to a less active analog. While the JAK3 potency increased, the compound was still over 70 fold selective for JAK3 over JAK2.

For 8d, the JAK1 activity was still not in the potency range we desired and additional modifications were sought. In this regard a set of analogs with different 6–membered ring heterocyclic and substituted phenyl compounds were examined (9a–d, Table 4). The 2-pyridyl compound 9a and 2,5-pyrazine analog 9b were considerably less potent than 8b. Note that neither compound has its terminal ring (pyridine for 9a or pyrazine for 9b) nitrogen atom in the para position (to the exocyclic amine) as it is for 8d. Compounds 9c and 9d that have phenyl or fluorophenyl rings devoid of pyridine-like nitrogen atoms are also much less potent than 8d. Thus it appears that the presence and trajectory of the pyridine nitrogen atom of 8b is very important for its potency as a JAK3 inhibitor.
We also examined replacing the 4-maleimide indole moiety with smaller groups and thus prepared phenyl and hydrogen replacement compounds (10a and b, Table 5). These compounds had greatly reduced activity when compared to 8d. Upon reflection, these results are not surprising when examining the X-ray structure of JAK3 complexed with 4 [Ref. 13 and Protein Data Bank (www.rcsb.org) file 3PJC]. In this structure the phenyl portion of the indole moiety of 4 makes four hydrophobic contacts with JAK3. These interactions would be lost or reduced when this indole is substituted by a phenyl or smaller groups. In addition, the NH of the indole of 4 (and presumably 8d) projects into bulk solvent possibly interacting with solvent water molecules and this interaction unavailable for 10a and b.

We then examined constrained analogs of 8d (Table 6, 11a–f), via transformation of the flexible ethyl amine side chain of 8d into piperidine, pyrrolidine or azetidine ring systems, in order reduce the number of unproductive conformations (from a JAK3 binding point of view). In addition, a set of compounds was prepared (11g–i) that further rigidified the analogs by also eliminating the oxygen atom linker with a direct bond to the aniline ring. Except for the azetidine analog 11i, which did show appreciable activity for JAK3 inhibition, this latter set exhibited no JAK inhibition activity at the highest doses tested (10 μM).

The piperidine set (11a, b) as well as the azetidine congener 11c, had activity that was 12–50 fold lower than lead 8d. However the pyrrolidine analog 11d showed improved potency over 8d in comparison to JAK3 potency and was approximately 100 fold selective for JAK3 over JAK2. The JAK1 potency had not improved however. Since this compound was a racemic mixture, the enantiomers of 11d were prepared. The S-isomer 11e was significantly more potent as a JAK 1/3 inhibitor than the R-enantiomer 11f.

Our simple overlay model was not able to predict this eutomer/disomer preference. Low energy conformations of either enantiomer 11e or f, when superpositioned with the JAK3 X-ray conformation of 4, appeared to overlay equally well with 4 (data not shown).

### Table 2
JAK inhibition of imidazole containing analogs

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Position</th>
<th>n=</th>
<th>IC₅₀ (nM)</th>
<th>JAK2/JAK3</th>
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<tbody>
<tr>
<td>7a</td>
<td>m</td>
<td>3</td>
<td>300</td>
<td>128 NA    78</td>
</tr>
<tr>
<td>7b</td>
<td>m</td>
<td>2</td>
<td>NA⁴ NA    1790 NA</td>
<td>6</td>
</tr>
<tr>
<td>7c</td>
<td>p</td>
<td>2</td>
<td>NA NA 278</td>
<td>7368 36</td>
</tr>
<tr>
<td>7d</td>
<td>p</td>
<td>3</td>
<td>8640 NA</td>
<td>1148 NA 9</td>
</tr>
<tr>
<td>7e</td>
<td>o</td>
<td>2</td>
<td>NA NA 9367</td>
<td>NA 1</td>
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</tbody>
</table>

*Compounds tested in triplicate, SEM generally ±20%.

### Table 4
JAK inhibition of 4-fluoro-pyridinyl amine replacement analogs

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>IC₅₀ (nM)</th>
<th>JAK2/JAK3</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>Ph</td>
<td>2174 11,080 576</td>
<td>NA</td>
</tr>
<tr>
<td>10b</td>
<td>H</td>
<td>NA⁴ NA</td>
<td>1790 NA 6</td>
</tr>
</tbody>
</table>

*Compounds tested in triplicate, SEM generally ±20%.

### Table 3
JAK inhibition of 4-fluoro-pyridinyl amine analogs

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>IC₅₀ (nM)</th>
<th>JAK2/JAK3</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>p</td>
<td>3 H H 1499</td>
<td>408 1380</td>
</tr>
<tr>
<td>8b</td>
<td>p</td>
<td>2 H H 2058</td>
<td>9687 201</td>
</tr>
<tr>
<td>8c</td>
<td>o</td>
<td>2 H H NA⁴</td>
<td>NA 1790</td>
</tr>
<tr>
<td>8d</td>
<td>m</td>
<td>2 H H 423</td>
<td>2130 29</td>
</tr>
<tr>
<td>8e</td>
<td>m</td>
<td>2 CH₃ H 1784</td>
<td>1637 179</td>
</tr>
<tr>
<td>8f</td>
<td>m</td>
<td>2 H CH₃ NA</td>
<td>490 46</td>
</tr>
</tbody>
</table>

*Compounds tested in triplicate, SEM generally ±20%.

### Table 5
JAK inhibition of 4-indole replacement analogs

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>IC₅₀ (nM)</th>
<th>JAK2/JAK3</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>Ph</td>
<td>2174 11,080 576</td>
<td>NA</td>
</tr>
<tr>
<td>10b</td>
<td>H</td>
<td>NA⁴ NA</td>
<td>1790 NA 6</td>
</tr>
</tbody>
</table>

*Compounds tested in triplicate, SEM generally ±20%.

NA = IC₅₀ > 10,000 nM.
In summary, we have designed novel, potent anilino-indolylmaleimide inhibitors of JAK3 based on a model constructed by superpositioning of JAK3 inhibitor 5, a PKC inhibitor we found to have JAK3 potency, increasing JAK1 potency, and maintaining selectivity over JAK2 and TYK2. Furthermore, compound 11d demonstrated good JAK3 potency as well as JAK2 and TYK2 selectivity to lead compound 5. These compounds were not evaluated as inhibitors for other kinases. Further compounds 11e like 3,13 showed poor cellular activity (inhibition of STAT5 phosphorylation induced by IL2 in CTLL cells, data not shown) providing further evidence that JAK1 inhibition may be also required for therapeutic intervention in RA. Since the potency of our series was modest for JAK1 inhibition, we declined to pursue this series further.

References and notes

26. (S)-3-{[1-(2-fluoro-pyridin-4-yl)pyrrolidin-3-yloxy]phenylamino}-4-(1H-indol-3-yl)pyrrole-2,5-dione (11e). (a) (S)-pyrrolidin-3-ol, 22 (500 mg, 5.75 mmol), 2,4-difluoropyridine (793 mg, 6.9 mmol) and DIPEA (0.5 mL) in DMF (5 mL) was heated at 60 °C for 20 h. The mixture was poured into 1 N NaOH solution (100 mL), extracted with ethyl acetate (2×). Combined extracts were washed with brine, dried over magnesium sulfate, and concentrated to give (S)-1-(4-fluoropyridin-2-yl)pyrrolidin-3-ol 23 (512 mg, 49% yield). (b) A mixture of 23 (91.5 mg, 0.5 mmol), 1-iodo-3-nitrobenzene (162 mg, 0.65 mmol), CuI (10 mg, 0.052 mmol), cesium carbonate (328 mg, 1.0 mmol) and 3,4,7,8-tetramethyl-1,10-phenanthroline (24 mg, 0.1 mmol) in THF (0.5 mL) was heated at 100 °C for 18 h. To the reaction mixture was added 20 mL of water and 20 mL of ethyl acetate. The resulting mixture was filtered through celite. The filtrate was separated; the aqueous layer was extracted with ethyl acetate (2×). Combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by normal phase flash chromatography (40–60% ethyl acetate/hexanes) and 4-((S)-3-(3-nitrophenoxy)pyrrolidin-1-yl)-2-fluoropyridine (40 mg, 26% yield) was used in the next step. The compound (75 mg, 0.247 mmol), and Pd/C (10%, 100 mg) in ethanol (5 mL) was hydrogenated at 40 psi for 2 h. Then the catalyst was removed by filtration. The filtrate was evaporated was evaporated to give 3-((S)-1-(2-fluoropyridin-4-yl)pyrrolidin-3-yloxy)benzeneamine 14v (61 mg, 50% yield). (c) The mixture of 14v (60 mg, 0.22 mmol) and 3-hydroxy-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (13) (16.7 mg, 0.073 mmol) in acetic acid (0.25 mL) was stirred at RT for 0.5 h, then heated at 100 °C for 3 h. The reaction mixture was diluted with 1 mL DMF, filtered, and the filtrate was purified by reverse phase chromatography to afford 11e (59 mg, 55% yield). 

1H NMR (300 MHz, DMSO-d6) δ 11.29 (d, J = 2.6 Hz, 1H), 10.69 (s, 1H), 9.12 (s, 1H), 7.76 (d, J = 5.9 Hz, 1H), 7.38 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.05 (d, J = 2.6 Hz, 1H), 6.98 (t, J = 7.6 Hz, 1H), 6.84 (dt, J = 21.5, 7.8 Hz, 2H), 6.63 (d, J = 8.5 Hz, 1H), 6.42–6.35 (m, 1H), 6.26 (dd, J = 7.8, 2.3 Hz, 1H), 6.11–6.02 (m, 2H), 4.02 (s, 1H), 3.25 (m, 1H), 3.19–3.00 (m, 2H), 1.89–1.62 (m, 2H). Mass (CI): C27H22FN5O3; Calcd Mass: 483.17, Found: 484.20 (M+).