<table>
<thead>
<tr>
<th>Title</th>
<th>Discovery of Oral VEGFR-2 Inhibitors with Prolonged Ocular Retention That Are Efficacious in Models of Wet Age-Related Macular Degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Meredith, Erik L.; Mainolfi, Nello; Poor, Stephen; Qiu, Yubin; Miranda, Karl; Powers, James; Liu, Donglei; Ma, Fupeng; Solovay, Catherine; Rao, Chang; Johnson, Leland; Ji, Nan; Artman, Gerald; Hardegger, Leo; Hanks, Shawn; Shen, Siyuan; Woolfenden, Amber; Fassbender, Elizabeth; Sivak, Jeremy M.; Zhang, Yiqin; Long, Debby; Cepeda, Rosemarie; Liu, Fang; Hosagrahara, Vinayak P.; Lee, Wendy; Tarsa, Peter; Anderson, Karen; Elliott, Jason; Jaffee, Bruce</td>
</tr>
<tr>
<td>Date</td>
<td>2015</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10220/39573">http://hdl.handle.net/10220/39573</a></td>
</tr>
<tr>
<td>Rights</td>
<td>© 2015 American Chemical Society. This paper was published in Journal of Medicinal Chemistry and is made available as an electronic reprint (preprint) with permission of American Chemical Society. The published version is available at: [<a href="http://dx.doi.org/10.1021/acs.jmedchem.5b01227">http://dx.doi.org/10.1021/acs.jmedchem.5b01227</a>]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law.</td>
</tr>
</tbody>
</table>
Discovery of Oral VEGFR-2 Inhibitors with Prolonged Ocular Retention That Are Efficacious in Models of Wet Age-Related Macular Degeneration

Erik L. Meredith,*† Nello Mainolfi,t,1 Stephen Poort,‡ Yubin Qiu,§ Karl Miranda,∥ James Powers,† Donglei Liu,† Fupeng Ma,† Catherine Solovay,† Chang Rao,‴ Leland Johnson,‴ Nan Ji,⊥ Gerald Artman,†,‡ Leo Hardegger,†,‡ Shawn Hanks,∥ Siyuan Shen, Amber Woolfenden,† Elizabeth Fassbender,† Jeremy M. Sivak,*‡ Yiqin Zhang,§,* Debby Long,‡ Rosemarie Cepeda,‡ Fang Liu,‡ Vinayak P. Hosagrahara,‡,§ Wendy Lee,§ Peter Tarsa,∥ Karen Anderson,‡ Jason Elliott,† and Bruce Jaffee‡

1Global Discovery Chemistry, Novartis Institutes for BioMedical Research, 100 Technology Square, Cambridge, Massachusetts 02139, United States
‡Ophthalmology, Novartis Institutes for BioMedical Research, 500 Technology Square, Cambridge, Massachusetts 02139, United States
§Metabolism and Pharmacokinetics, Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States
¶Chemical and Pharmaceutical Profiling, Novartis Institutes for BioMedical Research, 500 Technology Square, Cambridge, Massachusetts 02139, United States

ABSTRACT: The benefit of intravitreal anti-VEGF therapy in treating wet age-related macular degeneration (AMD) is well established. Identification of VEGFR-2 inhibitors with optimal ADME properties for an ocular indication provides opportunities for dosing routes beyond intravitreal injection. We employed a high-throughput in vivo screening strategy with rodent models of choroidal neovascularization and iterative compound design to identify VEGFR-2 inhibitors with potential to benefit wet AMD patients. These compounds demonstrate preferential ocular tissue distribution and efficacy after oral administration while minimizing systemic exposure.

INTRODUCTION

Age-related macular degeneration (AMD) is an eye disease that causes vision loss due to pathology in the macula, the area of the retina that enables sharp and detailed vision. AMD is responsible for the majority of cases of blindness and visual impairment in aged adults. In the wet form of AMD, choroidal blood vessels (choroidal neovascularization, CNV) sprout and expand into the submacular space leaking fluid and blood. This leads to retinal edema, scar tissue formation, and irreversible damage to the macula. The current approved therapies for wet AMD are the anti-VEGF-A antibody ranibizumab, the anti-VEGF-A aptamer pegaptanib, and the anti-VEGF-A and PLGF fusion protein aflibercept. All are delivered by intravitreal injection (i.v.t.), a procedure that carries a small, but significant, risk of blinding complications including endophthalmitis, retinal detachment, or uveitis. Ranibizumab and aflibercept are the most effective, stabilizing vision in 60% patients and significantly improving vision (gain of 3 lines of vision on an ETDRS reading chart) in approximately 1/3 of patients.1 Injections are administered monthly or bimonthly, which is a significant burden for patients and retinal doctors. Even with anti-VEGF therapy, wet AMD patients still have substantial visual deficit.2 We envisioned an opportunity for a less invasive wet AMD therapy based on an orally available inhibitor of the receptor tyrosine kinase (RTK) VEGFR-2 (KDR, Flt-1). An oral therapy may provide greater efficacy than intravitreal therapy by enabling more frequent dosing and greater target tissue exposure.

The role of VEGF-A in the regulation of angiogenesis is well established. Although new vessel growth and maturation are highly complex processes, requiring the sequential activation of a series of receptors by numerous ligands, VEGF-A signaling is a critical rate-limiting step.3 VEGF-A promotes growth of vascular endothelial cells and is also known to induce vascular leakage.4 Ocular VEGF-A levels are increased in preclinical models and human diseases of ocular angiogenesis including wet AMD,5,6 diabetic retinopathy, and retinal vein occlusions.7,8 VEGF-A binds to two related receptor tyrosine kinases, VEGFR-1 and VEGFR-2, which both have an extracellular domain consisting of 7 immunoglobulin-like domains, a single transmembrane region, and a consensus tyrosine kinase

Received: August 3, 2015
Published: November 15, 2015
Table 1. *In Vitro* Inhibition Data (KDR Biochemical; BaF3 Cellular) and *in Vivo* (% Inhibition of Mouse CNV) for Selected Compounds

<table>
<thead>
<tr>
<th>CMPD</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>KDR IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>BaF3 IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>mCNV % inhibition @ 10 mg/kg qd</th>
<th>CMPD</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>KDR IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>BaF3 IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>mCNV % inhibition @ 10 mg/kg qd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>17</td>
<td>--</td>
<td>44</td>
<td>33M</td>
<td></td>
<td></td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5a</td>
<td>N^+</td>
<td></td>
<td>13</td>
<td>4</td>
<td>45</td>
<td>33m</td>
<td></td>
<td></td>
<td>5</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>6a</td>
<td>N^+</td>
<td></td>
<td>8</td>
<td>11</td>
<td>67</td>
<td>33n</td>
<td></td>
<td></td>
<td>8</td>
<td>0.9</td>
<td>67</td>
</tr>
<tr>
<td>6b</td>
<td>N^+</td>
<td></td>
<td>5</td>
<td>66</td>
<td>92</td>
<td>33o</td>
<td></td>
<td></td>
<td>&lt;3</td>
<td>0.5</td>
<td>43</td>
</tr>
<tr>
<td>33a</td>
<td></td>
<td></td>
<td>22</td>
<td>10</td>
<td>87</td>
<td>33p</td>
<td></td>
<td></td>
<td>8</td>
<td>0.9</td>
<td>48</td>
</tr>
<tr>
<td>33b</td>
<td></td>
<td></td>
<td>18</td>
<td>81</td>
<td>0</td>
<td>33q</td>
<td></td>
<td></td>
<td>30</td>
<td>40</td>
<td>83</td>
</tr>
<tr>
<td>33c</td>
<td></td>
<td></td>
<td>24</td>
<td>60</td>
<td>96</td>
<td>33r</td>
<td></td>
<td></td>
<td>30</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>33d</td>
<td></td>
<td></td>
<td>23</td>
<td>109</td>
<td>58</td>
<td>33s</td>
<td></td>
<td></td>
<td>9</td>
<td>59</td>
<td>83</td>
</tr>
<tr>
<td>33e</td>
<td></td>
<td></td>
<td>7</td>
<td>--</td>
<td>0</td>
<td>33t</td>
<td></td>
<td></td>
<td>114</td>
<td>167</td>
<td>58</td>
</tr>
<tr>
<td>33f</td>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>81</td>
<td>33u</td>
<td></td>
<td></td>
<td>22</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>33g</td>
<td></td>
<td></td>
<td>29</td>
<td>0.4</td>
<td>68</td>
<td>33v</td>
<td></td>
<td></td>
<td>41</td>
<td>26</td>
<td>98</td>
</tr>
<tr>
<td>33h</td>
<td></td>
<td></td>
<td>16</td>
<td>118</td>
<td>41</td>
<td>33w</td>
<td></td>
<td></td>
<td>9</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>33i</td>
<td></td>
<td></td>
<td>17</td>
<td>--</td>
<td>0</td>
<td>33x</td>
<td></td>
<td></td>
<td>14</td>
<td>6</td>
<td>90</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are the mean of at least two experiments (IC<sub>50</sub>, nM).<sup>b</sup>Compounds dosed q.d. orally as 0.5% CMC/0.1% Tween 80.

Sequence that is interrupted by a kinase-insert domain. VEGFR-2 (KDR or Flt-1) is implicated in all aspects of normal and pathological vascular endothelial cell biology. VEGFR-2 is the major mediator of the mitogenic, angiogenic,
and permeability-enhancing effects of VEGF-A. VEGFR-2 null mice die in utero between days 8.5−9.5 and are characterized by a lack of vasculogenesis and failure to develop organized blood vessels.10 Upon VEGF-A binding, VEGFR-2 undergoes dimerization and ligand-dependent tyrosine phosphorylation in intact cells, resulting in a mitogenic, chemotactic, and survival signal.11 Systemic inhibition of the VEGF pathway is known to cause on-target side effects including hypertension, proteinuria, bleeding, thromboembolism, and gastrointestinal perforation.12 Our strategy to mitigate the risk of systemic VEGF inhibition was to identify compounds with preferential distribution and retention in ocular tissues (retina and retinal pigment epithelium/choroid) compared to the plasma.

There are a number of approved orally administered VEGFR-2 inhibitors for oncology indications and several have been investigated in wet AMD.13−15 In a published case study, treatment with oral sorafenib correlated with reduced retinal edema and increased visual acuity.16,17 More recently, orally dosed pazopanib is reported to improve vision, reduce edema, and reduce neovascularization in wet AMD patients.18 Despite the fact the doses used were markedly less than those in oncology indications, systemic side effects were still observed. Thus, a compound that more selectively targets ocular tissues than marketed oncology drugs would potentially be an effective and safer therapy for wet AMD patients.

To achieve our objective of identifying compounds with preferential ocular distribution, we understood that relatively high-throughput in vivo models of ocular angiogenesis would be needed to establish both ocular efficacy and tissue distribution relationships. The primary in vivo models utilized were laser-induced choroidal neovascularization (CNV) in mice and in rats. Briefly, photocoagulating green laser pulses are applied to the eye in order to cause a focal rupture of Bruch’s membrane, an extracellular matrix between the retina and choroid. Rupture of Bruch’s membrane induces production of local inflammatory factors and VEGF resulting in choroidal neovascularization (CNV). CNV formation in response to laser injury has been demonstrated in humans, monkeys, pigs, and rodents and is a VEGF-dependent pathology.19 Evaluation of several internal VEGFR-2 kinase inhibitors in the mouse CNV model provided 2 as a promising starting point for chemistry optimization. The compound demonstrated a modest level of inhibition (44%) but had poor aqueous solubility (<5 μM at pH 6.8). Thus, we undertook a strategy to identify compounds with greater efficacy and improved ADME properties.

Chemistry. The preparation of monocyclic pyrimidine analogues such as 6 was carried out as described in Scheme 1. Coupling of indole 2 with pyrimidine 321 was achieved by treatment with DBU. Formation of the urea was then accomplished by trapping the lithium anion of the indole with the desired phenylisocyanates. Removal of the benzyl protecting group with TFA provided alcohol 5, which was readily converted to methylamine 6 by conversion first to the mesylate and then displacement with methylamine.

The bicyclic pyrimidine intermediates 8 and 10 were accessible from the corresponding β-ketoesters 7 and 9.
Treatment of each β-ketoester 7 and 9 with formamidine acetate provided the corresponding hydroxypyrimidine, which then underwent a protecting group swap from benzyl to Boc. Conversion to the chloropyrimidines 8 and 10 was then accomplished by reaction with carbon tetrachloride and triphenylphosphine.

The 8-methyl substituted bicyclic pyrimidine 15 was prepared as outlined in Scheme 3. The β-ketoester 14 was converted to the corresponding Boc protected chloropyrimidine by analogy to that of 8 and 10.

The chiral chloropyrimidines 21 and 22 were prepared starting from (S)-α-methylbenzylamine, which underwent reductive amination with 16 and then ethyl glyoxylate to provide diester 18 (Scheme 4). Claisen condensation of 18 then provided diastereomers 19 and 20, which were separated by flash chromatography. Conversion of each diastereomer 19 and 20 to the corresponding chloropyrimidines 21 and 22 was achieved under the same conditions as those described above.

The 6,5-bicyclic pyrimidine 24 was prepared by condensation of the commercially available β-ketoester 23 with formamidine acetate (Scheme 5). Construction of the methyl substituted 6,5-bicyclic pyrimidine was as described in Scheme 6. In each of these cases, the hydroxy pyrimidines were used in the subsequent coupling steps as the chloro derivatives were found to be both difficult to prepare and use due to instability.

Coupling of the bicyclic pyrimidines 10, 15, 21, and 22 to the hydroxindole was achieved by treatment with DBU to give the corresponding derivatives 30 (Scheme 7). The 6,5-bicyclic pyrimidines 24 and 28 were efficiently coupled to 29 with PyBop and DBU. Formation of the desired urea analogues 33 was achieved by treatment of the sodium anion of 30 with either a preformed phenylcarbamate or a commercially available isocyanate. The aminopyrazoles and aminoisoxazoles 31 that were not commercially available were prepared as described previously. The Boc protecting group was then removed upon treatment with TFA to provide the desired compounds 33 for testing against VEGFR-2.

**Scheme 4**

```
Reagents and conditions: (i) 16, 17, NaBH(OAc)_3, DCE; (ii) ethyl glyoxylate, NaBH(OAc)_3, DCE; (iii) tBuOK, toluene, 50–70%, 3 steps; (iv) formamidine acetate, EtONa, EtOH, reflux, 93%; (v) Pd/C, Boc_2O, EtOH, 80%, 2 steps; (vi) CCl_4, PPh_3, DCE, reflux, 51%.
```

**Scheme 5**

```
Reagents and conditions: (i) formamidine acetate, EtONa, EtOH, reflux, 24%.
```

**Scheme 6**

```
Reagents and conditions: (i) NaH, toluene, 0 °C to rt; (ii) formamidine acetate, EtONa, EtOH, reflux, ~ 20%.
```

**Scheme 7**

```
Reagents and conditions: (i) 10, 15, 21, or 22, DBU, CH_3CN, reflux, 90%; (ii) 24 or 28, PyBop, acetonitrile, DBU, 64%; (iii) 25, phenyl chloroformate, pyridine, 86%; (iv) 32 or R_2NCO, NaH, THF, 0 °C–rt, 50%; (v) TFA, DCM, 92%.
```
As we sought to identify compounds that selectively distributed to the ocular tissues, we did not apply the usual in vitro PK-ADME filters prior to in vivo evaluation. Priority was given to compounds that had demonstrated good aqueous solubility, yet generally those with good in vitro potency were selected for evaluation in the mouse laser CNV model (C57BL/6) at a single dose of 10 mg/kg (p.o.). This screening dose was selected based on early efficacy studies with existing VEGFR-2 inhibitors, such as 1, and was not expected to cause any acute side effects that would compromise a given study. Compounds were formulated as suspensions (0.5% methyl cellulose, Tween 80, and water) to better inform our understanding of their intrinsic ADME properties. Each compound was dosed starting the day of laser application and continued daily with the last dose given on day 6 and CNV area measurement on day 7. Comparison of CNV area in animals dosed with compound or placebo was used to establish percent inhibition.

Compounds exhibiting >60% inhibition at the 10 mg/kg screening dose were further evaluated in mouse CNV dose–response studies to establish ED90 and ED90 relationships. Compounds with an ED90 < 20 mg/kg in the mouse CNV assay were then characterized in the rat laser CNV model (Brown Norway). To understand the correlation between efficacy and exposure, the distribution profiles for select compounds were determined by orally dosing Brown Norway rats and measuring time course drug exposures in the plasma and ocular tissues (retina and retinal pigment epithelium/choroid/sclera complex). Further understanding of the ADME profiles for select compounds was accomplished by discrete i.v./p.o. PK studies in Brown Norway rats.

### RESULTS

Early on, we recognized the need to improve on the aqueous solubility of compound 1. It was anticipated that greater aqueous solubility would provide increased oral bioavailability and as a consequence better efficacy. X-ray cocrystal structures of related VEGFR-2 inhibitors indicated that incorporation of ionizable groups on the pyrimidine should be possible. An important early finding was that the aminopyrimidine of 1 was not required for potency as 5a proved to be equipotent in the biochemical assay and have similar efficacy in the mouse CNV model (Table 1; 45% inhibition). A basic amine was introduced to give compound 6a, which demonstrated similar potency in vitro and importantly increased efficacy in vivo (67% inhibition).

As anticipated, 6a had improved aqueous solubility (Table 2; 64 μM) relative to that of 1. Incorporation of a fluoro substituent in the 4-position of the aniline as in 6b provided a boost in efficacy as the compound showed 92% inhibition of neovascularization in the mouse at the 10 mg/kg dose.

Collectively, these compounds demonstrated the potential for optimization through inclusion of the basic amine moiety; however, 6b was found to have an undesirable level of activity on the hERG channel (Table 3; IC50 = 5800 nM). To optimize in vivo efficacy while balancing hERG activity and aqueous solubility, we focused our efforts on the basic amine region and the urea side chain of 6b. Our exploration centered on altering both the pKa and the steric environment of the amine as well as reducing the lipophilicity of the urea side chain. It was thought that a combination of these changes would provide the desired efficacy while reducing hERG liability.

Rigidification of the amine side chain by incorporation into the 6,6-bicyclic pyrimidine 33a was well tolerated as it gave

---

**Table 2. Comparison of Aqueous Solubility for Selected VEGFR-2 Inhibitors**

<table>
<thead>
<tr>
<th>cmpd</th>
<th>aq sol pH 6.8 (μM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>6a</td>
<td>64</td>
</tr>
<tr>
<td>33a</td>
<td>84</td>
</tr>
<tr>
<td>33f</td>
<td>&lt;5</td>
</tr>
<tr>
<td>33j</td>
<td>190</td>
</tr>
<tr>
<td>33k</td>
<td>833</td>
</tr>
<tr>
<td>33l</td>
<td>682</td>
</tr>
<tr>
<td>33m</td>
<td>252</td>
</tr>
<tr>
<td>33n</td>
<td>450</td>
</tr>
<tr>
<td>33o</td>
<td>489</td>
</tr>
<tr>
<td>33q</td>
<td>167</td>
</tr>
<tr>
<td>33r</td>
<td>126</td>
</tr>
<tr>
<td>33s</td>
<td>20</td>
</tr>
<tr>
<td>33t</td>
<td>232</td>
</tr>
<tr>
<td>33v</td>
<td>110</td>
</tr>
<tr>
<td>33w</td>
<td>37</td>
</tr>
</tbody>
</table>

*Aqueous high-throughput equilibrium solubility (see ref 26).

**Table 3. Comparison of hERG Activity for Selected VEGFR-2 Inhibitors**

<table>
<thead>
<tr>
<th>cmpd</th>
<th>hERG IC50 (nM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6b</td>
<td>5800</td>
</tr>
<tr>
<td>33a</td>
<td>8900</td>
</tr>
<tr>
<td>33c</td>
<td>10700</td>
</tr>
<tr>
<td>33g</td>
<td>15600</td>
</tr>
<tr>
<td>33m</td>
<td>&gt;30000</td>
</tr>
<tr>
<td>33n</td>
<td>27400</td>
</tr>
<tr>
<td>33q</td>
<td>7000</td>
</tr>
<tr>
<td>33r</td>
<td>11100</td>
</tr>
<tr>
<td>33s</td>
<td>23500</td>
</tr>
<tr>
<td>33v</td>
<td>&gt;30000</td>
</tr>
</tbody>
</table>

*Automated Q-patch assay.

87% inhibition in the mouse CNV model with modest aqueous solubility (84 μM). However, no substantial reduction in hERG activity was realized (IC50 = 8900 nM). Intriguingly, the isomeric 6,6-bicyclic pyrimidine 33b provided no significant inhibition in vivo despite having good potency in the enzymatic and cellular assays.

To further probe the sensitivity of this region to substitution, the enantiomeric methyl analogues 33c and 33d were prepared. These compounds again highlighted a similar pattern of disconnect between the in vitro and the in vivo efficacy as 33c, the (S)-enantiomer, demonstrated greater efficacy despite similar in vitro potency to the (R)-enantiomer 33d. Acetylation of the amine as in 33e caused a complete loss of efficacy in the mouse model, while retention of the basic amine as in the ethyl derivative 33f provided 81% inhibition. However, the tertiary amine 33f reduced the aqueous solubility (<5 μM).

Reduction of amine pKa was achieved with the 6,5-bicyclic pyrimidine 33g. This compound was found to be efficacious (68% inhibition) in the mouse model and demonstrated reduced hERG activity (IC50 = 15600 nM). In this instance, however, the addition of a methyl group to the bicyclic as in 33h and 33i attenuated the efficacy.

The pyridyl analogue 33j was designed to reduce the potency in the biochemical (280 nM) and cell (1922 nM) assays...
was attenuated, it demonstrated the potential benefit through further optimization to identify more preferred heteroaryl groups as there was a favorable impact on aqueous solubility (190 \( \mu \)M). Indeed, further design of heteroaryl replacements led to pyrazoles 33k and 33l that provided very good \textit{in vitro} potency (cell IC\(_{50}\) 3 nM and 1 nM, respectively). While the initial pyrazole analogues 33k and 33l provided no inhibition in the mouse CNV model, they did have markedly improved aqueous solubility (833 and 682 \( \mu \)M, respectively). Further design and optimization led to the discovery that the cyclopropylmethyl analogue 33m provided 64\% inhibition. The close structural similarities among these analogues again provide a dramatic demonstration of the subtle effects realized only with \textit{in vivo} SAR. Pyrazole 33m had good aqueous solubility (252 \( \mu \)M) and minimal hERG activity with an IC\(_{50}\) > 30,000 nM. This was an important finding as it demonstrated the ability to reduce hERG activity without alteration of the amine basicity.

Combining the methyl substituted 6,6-bicyclic pyrimidine with the cyclopropymethyl pyrazole as in 33n gave good potency in the cellular assay (0.9 nM), efficacy in the mouse (67\% inhibition), good aqueous solubility (450 \( \mu \)M), and minimal hERG activity (IC\(_{50}\) 27,400 nM). In this case, as shown above, the opposite enantiomer 33p and the 6,5-bicyclic analogue 33o showed reduced efficacy \textit{in vivo} despite similar potency \textit{in vitro}.

Further exploration of aniline replacements revealed that the isoxazole (e.g., 33q and 33r with 83\% and 96\% inhibition, respectively) provided even greater efficacy than the corresponding pyrazole analogues; however, they also had greater hERG activity. It was also found that the 3-aminooxazoles analogues (e.g., 33r) were more active \textit{in vivo} than the isomeric 5-aminooxazoles (e.g., 33q). Similar to the pyrazole analogues, inclusion of the methyl group in 6,5-pyrimidine did not give a favorable increase in activity (33u), which was again in contrast to the 6,6-bicyclic pyrimidines. Modification of the isoxazole substitution from \textit{t}-butyl to cyclopropyl 33v or isopropyl 33w provided maximal inhibition in the mouse model (98 and 96\% inhibition, respectively). Isoxazole 33v also showed minimal hERG activity with an IC\(_{50}\) > 30,000 nM in combination with good aqueous solubility (110 \( \mu \)M).

Dose–response studies in the mouse and rat were conducted for the preferred compounds identified from the mouse \textit{in vivo} single dose screen (Table 4). In the mouse laser CNV model, the isoxazole derivative 33v provided the greatest efficacy on a per dose basis with an ED\(_{50}\) of 4.8 mg/kg, while its corresponding aniline analogue 33g provided an ED\(_{50}\) of 31 mg/kg. In contrast, the pyrazole analogue 33n was found to have a higher ED\(_{50}\) (19.6 mg/kg) than its corresponding aniline derivative 33c in the mouse CNV. Both the nonmethylated 33m and the methylated 33n provided a similar dose–response in the mouse model. Further characterization of 33n, 33v, and 33m in the rat laser CNV model again demonstrated that on a per dose basis 33v was more efficacious than either 33m or 33n.

As stated above, our key objective was to identify VEGFR-2 inhibitors that provided good efficacy in the mouse and rat laser CNV models following oral dosing and demonstrated distribution to and retention in the ocular tissues relative to the plasma. Discrete oral PK studies in Brown Norway rats were conducted with preferred compounds. Exposure in plasma, retina, and posterior eye cup (retinal pigment epithelium/choroid sclera complex = posterior eye cup) were assessed at 7 time points after dosing between 0 and 24 h. In Table 5, the dose normalized (DN) exposures for 3 compounds are detailed. It can readily be seen that 33v has significantly higher plasma exposure (DN\(_{\text{max}}\) and DNAUC) than either 33m or 33n. In the retina, 33n and 33v had similar exposure, while 33m was measurably lower. In the PEC, there was even greater contrast among the three compounds with the exposure decreasing in order from 33v > 33n > 33m. The data from the ocular tissues indicate a strong preference for the retention of each of the compounds in those tissues versus retention in the plasma. This is most easily noted by comparison of the plasma/retina/PEC dose normalized concentrations at 24 h (33n, 1.6/122/1067 nM; 33v, 6/65/3334 nM; 33m, 0.7/13/102 nM). The above data demonstrate that it is possible to identify compounds with preferential distribution to the ocular tissues and reduced plasma exposure. The advantage of such distribution in mitigating systemic side effects will only be borne out in toxicology studies as it was not expected that our efficacy models would address this. However, it is worth noting that the approved VEGFR-2 inhibitor pazopanib, described above, has been reported to provide 93% inhibition of laser-induced CNV in the mouse model when dosed orally at 100 mg/kg twice daily and that dose when given for 7 d produced a plasma exposure of \( \sim \)13 \( \mu \)M at 16 h post the last dose.\(^{27}\)

The i.x./p.o. PK parameters of 33n, 33v, and 33m provide further details regarding systemic exposure and clearance (Table 6). In accord with the ocular PK study above, 33n shows a higher rate of clearance from the plasma (165 mL/min/kg), while 33v provided much lower clearance (6 mL/min/kg). All three of these compounds were found to have good plasma exposure following oral dosing (F\% > 50). The plasma and ocular PK data highlight the limitation of optimizing compounds with limited \textit{in vivo} efficacy measurements or an over reliance on \textit{in vivo} PK as 33m would not have been preferred either from plasma or ocular PK, yet the compound is efficacious at reducing laser-induced CNV.

In a selectivity panel consisting of 62 kinases, 33n and 33v were found to have activity <1 \( \mu \)M against several related kinases [IC\(_{50}\) (\( \mu \)M) 33n, Ret 0.066, PDGFR\( \alpha \) 0.15, and c-Kit 0.15, PDGFR\( \alpha \) 0.15 \( \mu \)M]. This is consistent with the observed \textit{in vivo} efficacy being primarily driven by VEGFR-2 inhibition.

### CONCLUSIONS

Predicting ocular efficacy based on \textit{in vitro} potency and \textit{in vivo} exposure remains a challenge in ocular drug discovery. This is in part evidenced by the high number of potent VEGFR-2

### Table 4. Dose–Response Inhibition of Laser Induced Choroidal Neovascularization for Selected VEGFR-2 Inhibitors

<table>
<thead>
<tr>
<th>cmpd</th>
<th>mouse ( \text{ED}_{50} ) (mg/kg)</th>
<th>rat ( \text{ED}_{50} ) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33c</td>
<td>3.6</td>
<td>8.5</td>
</tr>
<tr>
<td>33g</td>
<td>6.9</td>
<td>31</td>
</tr>
<tr>
<td>33n</td>
<td>3.4</td>
<td>19.6</td>
</tr>
<tr>
<td>33v</td>
<td>1.8</td>
<td>4.8</td>
</tr>
<tr>
<td>33m</td>
<td>7.8</td>
<td>25.3</td>
</tr>
</tbody>
</table>

\(^{4}\)Compounds dosed q.d. orally as 0.5% MC/0.1% Tween 80, WFI 7 days. \(^{5}\)Compounds dosed q.d. orally as 0.5% CMC/0.1% Tween 80, WFI 14 days.
inhibitors described herein, which provide little or no efficacy in the mouse laser CNV model. The challenge is more clearly illustrated by molecules 33m, 33n, and 33v, which provide similar in vivo efficacy despite substantially different tissue distribution profiles. The reasons for these disconnects are not fully understood at this time. We believe melanin binding plays a key role not only in the preferential distribution of the compound to the ocular tissue but also in the differing correlations between ocular exposure and efficacy in the CNV models. In part, the answer may come from melanin binding studies that adequately provide the kinetic binding profile of the molecules. Differential kinase selectivity may provide another hypothesis to explain the exposure—efficacy disconnect, although data collected against a kinase panel indicate that the molecules are reasonably selective for VEGFR-2 and have similar broad profiles.

Despite the inability to a priori predict ocular efficacy based on plasma PK and potency, our use of relatively high-throughput in vivo screening in a mouse laser CNV model facilitated the generation of clear and actionable in vivo SAR. Through optimization of one particular series of VEGFR-2 inhibitors, described herein, multiple promising compounds were identified that effectively reduced choroidal neovascularization and provided preferential distribution to the ocular tissues. The observations made with 33m, 33n, and 33v are a clear demonstration of the ability to identify VEGFR-2 inhibitors with markedly higher ocular exposure than that in the plasma. Selective distribution to the target tissue of interest, the eye in this case, may provide an advantage in minimizing systemic side effects. This is of particular interest as the on-target systemic toxicity of VEGFR-2 inhibitors has been well established and thus may be a barrier to systemic use of such therapy in the treatment of AMD. This data may support the potential clinical utility of systemically administered VEGFR-2 inhibitors with preferential ocular distribution as a therapy for wet AMD. A similar strategy may be applicable to other disease indications that would benefit from local drug distribution to limit systemic toxicity.

### Experimental Section

#### Chemistry: General

NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. The following abbreviations are used to denote signal patterns: s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad. Flash chromatography was conducted using grade 60–230–400 mesh silica gel from Fisher Chemical (S825-1) or by utilizing the CombiFlash Companion from Teledyne Isco, Inc. and RediSep Rf disposable normal phase silica gel columns (4–300 g). Thin layer chromatography was performed using 2.5 × 7.5 cm glass-backed TLC Silica Gel 60 F254 plates from EMD Chemicals, Inc. (15341-1) and visualized by UV light. HPLC purifications were performed on a Gilson preparative HPLC system controlled by Unipoint software using X-Bridge Phenyl, C8, C18, or RP18 30 × 50 mm columns with 5 μm particle size. The purity of all compounds was ≥95%, unless otherwise noted. Low-resolution mass spectra were recorded using an Agilent 1100 series LC-MS spectrometer.

5-(6-Benzyloxymethyl-pyrimidin-4-yloxy)-1H-indole (4). 5-Hydroxy indole 2 (5 g, 37.6 mmol) was added in one portion to a solution of 4-(benzylxoxymethyl)-6-chloroprimidine 3 (8.81 g, 37.6 mmol) and DBU (5.66 mL, 37.6 mmol) in ACN (107 mL) at room temperature. This was stirred overnight for 20 h. The reaction was concentrated and purified via FCC (12–100% EtOAc/heptane) to obtain the title compound (3.97 g, 28.3 mmol, 75% yield). MS (ESI) m/z 447.0 (M + 1); 1H NMR (400 MHz, DMSO-d6) δ 6.11–11.44 (m, 1 H), 8.58–8.82 (m, 1 H), 7.41–7.51 (m, 2 H), 7.34–7.39 (m, 1 H), 7.21–7.32 (m, 5 H), 6.88–6.94 (m, 2 H), 6.62–6.65 (m, 1 H), 4.52–4.64 (m, 4 H).

5-(4-Hydroxymethyl-pyrimidin-4-yloxy)-1-carboxylic Acid (4-Fluoro-3-trifluoromethyl-phenyl)-amide (5). To NaNH (255 mg, 6.37 mmol) in 40 mL of DMF at 0 °C under nitrogen was added a solution of 5-(4-benzylxoxymethyl)-4-aryl indole (960 mg, 2.90 mmol) in 5 mL of DMF and stirred at 0 °C for 0.5 h. The reaction was then cooled to −50 °C, followed by the addition of 1-flouro-4-isocyanato-2-(trifluoromethyl)benzene (0.454 mL, 3.19 mmol) in 5 mL of DMF. The cold bath was removed, and the reaction allowed to warm to RT. After 3 h, the reaction was quenched with NH4Cl, extracted 2× EtOAc, and the combined organics washed with H2O (3×), brine, dried over Na2SO4 and concentrated. The residue was then purified via FCC (1–50% EtOAc/CH2Cl2) to obtain...
Journal of Medicinal Chemistry

5-(6-((benzoyl)oxy)methyl)pyrimidin-4-yl)oxy)-N-(4-fluoro-3-(trifluoromethyl)phenyl)-1H-indole-1-carboxamide (1.48 g, 2.76 mmol) in TFA (15 mL) was heated at 100 °C for 4 h before being filtered. The filtrate was used in the next step without further purification. MS (ESI) m/z 525.0 (M + 1).

To a solution of 5-(6-methanesulfonyl-methyl-pyrimidin-4-yl)-indole-1-carboxylic acid (1.26 g, 2.67 mmol) and methanesulfonic anhydride (240 mg, 1.4 mol) in THF (15 mL) was added pyridine (0.2 mL). The mixture was stirred at rt for 0.5 h, then filtered, and concentrated under reduced pressure. The residue was diluted with EtOAc and washed with water and brine. The organic layer was removed, dried over anhydrous Na2SO4, and concentrated. The crude residue was used without further purification. MS (ESI) m/z 250.2 (M + 1).

4-Chloro-5,8-dihydro-6H-pyrido[3,4-d]pyrimidine-7-carboxylic acid tert-Butyl Ester (8). To a solution of ethyl 4-(2-ethoxy-2-oxoethyl)((S)-1-phenylethyl)amino)pentanoate (2.8 g, 6.7 mmol) in toluene (500 mL) was added ethyl glyoxylate (84 g, 412 mmol, 50% toluene). The reaction was stirred at room temperature for 18 h. The mixture was then partitioned between DCM and saturated aqueous NaHCO3. The organic layer was removed, and the aqueous layer was extracted further with DCM (2 × 200 mL) and stirred at rt for 4 h. The reaction was then partitioned between DCM and saturated aqueous NaHCO3. The organic layer was removed, dried over anhydrous Na2SO4, and concentrated in vacuo. The crude residue was used without further purification. MS (ESI) m/z 252.0 (M + 1).

4-Chloro-5,8-dihydro-6H-pyrido[3,4-d]pyrimidine-7-carboxylic acid tert-Butyl Ester (8). To a solution of ethyl 4-(2-ethoxy-2-oxoethyl)((S)-1-phenylethyl)amino)pentanoate (2.8 g, 6.7 mmol) in toluene (500 mL) was added ethyl glyoxylate (84 g, 412 mmol, 50% toluene). The reaction was stirred at room temperature for 18 h. The mixture was then partitioned between DCM and saturated aqueous NaHCO3. The organic layer was removed, and the aqueous layer was extracted further with DCM (2 × 200 mL). The combined organic layers were then dried over anhydrous Na2SO4, filtered, and concentrated under reduced pressure. The crude diastereomeric mixture of ethyl 4-((2-ethoxy-2-oxoethyl)((S)-1-phenylethyl)amino)pentanoate (18) was used without further purification. MS (ESI) m/z 336.4 (M + 1).

25-((2-ethoxy-2-oxoethyl)((S)-1-phenylethyl)amino)pentanoate (20). To a solution of ethyl 4-((2-ethoxy-2-oxoethyl)((S)-1-phenylethyl)amino)pentanoate (44.5 g, 133 mmol) in toluene (500 mL) was added KOtBu (37.2 g, 332 mmol). After stirring for 3 h at room temperature, the mixture was then partitioned between DCM and saturated aqueous NH4Cl. The organic layer was removed, and the aqueous layer was extracted further with DCM (2 × 200 mL). The combined organic layers were then dried over anhydrous Na2SO4, filtered, and concentrated under reduced pressure. The crude diastereomeric mixture of ethyl 4-((2-ethoxy-2-oxoethyl)((S)-1-phenylethyl)amino)pentanoate (18) was used without further purification. MS (ESI) m/z 336.4 (M + 1).
residue was purified via FCC (2.5% EtOAc/heptane) to give both of the title diastereomers ([19, 20.6 g, 54%]; [20, 7.5 g, 19%]. Analytical data for 19: MS (ESI) m/z 290.2 (M + 1); 1H NMR (400 MHz, chloroform-d) δ 1.18 (s, 1 H), 1.77−1.87 (m, 5 H, 4), 1.96−2.42 (m, 2 H), 2.70 (dd, J = 15.5, 3.7 Hz, 1 H), 2.93 (dd, J = 17.2, 6.0 Hz, 1 H), 2.99 (dd, J = 17.2, 6.0 Hz, 1 H), 3.97 (dd, J = 17.2, 5.7 Hz, 1 H), 7.30 (m, 5 H), 4.16−4.29 (m, 2 H), 4.86−4.89 (m, 1 H), 4.91−4.94 (m, 1 H), 8.62 (d, J = 4.0 Hz, 1 H), 7.41−7.44 (m, 2 H), 7.35 (d, J = 2.2 Hz, 1 H), 6.92 (d, J = 8.8 Hz, 1 H), 4.44 (t, J = 2.15 Hz, 1 H), 4.50−4.61 (m, 3 H), 4.51 (s, 1 H), 1.49−1.51 (m, 9 H), 0.90 (d, J = 6.8 Hz, 3 H). A solution of (S)-tert-butyl 6-methyl-4-oxo-3,4,5,6-tetrahydropyrido[3,4-d]pyrimidine-7(8H)-carboxylate (21.5 g, 71 mmol) in THF (200 mL) was heated at 90 °C for 3 h. At that time, the solvent was removed under reduced pressure, and the residue was taken up in EtOH and washed with sat aq NH₄Cl. The aqueous layer was further extracted with EtOAc, and the combined organic layers were then dried over sodium sulfate, filtered, and concentrated to give (S)-6-methyl-7-(S)-1-phenylethyl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4(3H)-one (20.6 g, 74 mmol), ammonium formate (243.4 g, 371 mol), and BOC-anhydrolysin (48.6 g, 223 mmol), in EtOH (300 mL), was added 20% Pd(OH)₂/C (8.19 g, puri 284.1 (M + H). 1H NMR (400 MHz, CDCl₃) δ 1.91−1.94 (m, 1 H), 1.97−1.99 (m, 1 H), 2.48−2.50 (m, 1 H), 2.73−2.75 (m, 1 H), 2.92−2.95 (m, 1 H), 3.67−3.70 (m, 1 H), 4.48−4.50 (m, 1 H), 4.63−4.66 (m, 1 H), 4.99−5.01 (m, 1 H), 4.95−5.01 (m, 1 H), 6.96−6.99 (m, 1 H), 7.36−7.39 (m, 5 H), 4.13 (d, J = 6.8 Hz, 1 H), 6.76−7.00 (m, 3 H). The reaction was stirred at 90 °C for 3 h. The reaction mixture was then filtered through Celite pad, and the filtrate was concentrated. The residue was purified via FCC (5−5% MeOH/DCM) to give (S)-tert-butyl 6-methyl-4-oxo-3,4,5,6-tetrahydropyrido[3,4-d]pyrimidine-7(8H)-carboxylate (15.7 g, 80%). MS (ESI) m/z 266.1 (M + H); 1H NMR (400 MHz, chloroform-d) δ 0.70 (s, 1 H), 8.09 (s, 1 H), 4.85−4.66 (m, 2 H), 4.03 (d, J = 19.18 Hz, 1 H), 2.70 (dd, J = 17.2, 6.0 Hz, 1 H), 2.59 (d, J = 17.2, 6.0 Hz, 1 H), 1.49 (s, 9 H), 0.10 (d, J = 6.9 Hz, 3 H). A solution of (S)-tert-butyl 6-methyl-4-oxo-3,4,5,6-tetrahydropyrido[3,4-d]pyrimidine-7(8H)-carboxylate, (15.1 g, 57.1 mmol), triphenylphosphine (29.9 g, 114 mmol), and carbon tetrachloride (165.6 mL, 171 mmol) in 1,2-DCE (300 mL) was heated at 70 °C for 3 h. At that time, the solution was concentrated in vacuo, and the residue was separated via FCC (5−60% EtOAc/heptane) to give (S)-tert-butyl 4-chloro-6-methyl-5,8-dihydropyrido[3,4-d]pyrimidine-7(8H)-carboxylic acid (15.7 g, 80%). MS (ESI) m/z 283.1 (M + H); 1H NMR (400 MHz, chloroform-d) δ 0.78 (s, 1 H), 8.73 (s, 1 H), 5.13 (s, 1 H), 4.99 (d, J = 19.7 Hz, 1 H), 4.84 (s, 1 H), 4.25 (d, J = 19.7 Hz, 1 H), 2.99 (dd, J = 17.1, 5.9 Hz, 1 H), 2.74 (d, J = 17.1, 5.9 Hz, 1 H), 1.48 (s, 9 H), 1.11 (d, J = 7.0 Hz, 3 H). (R)-tert-Butyl 4-Chloro-6-methyl-5,6-dihydropyrido[3,4-d]pyrimidine-7(8H)-carboxylate (22). Prepared as described for 21. MS (ESI) m/z 284.0 (M + H); 1H NMR (400 MHz, chloroform-d) δ 8.81 (s, 1 H) 4.99 (d, J = 19.70 Hz, 1 H) 4.86 (br. s, 1 H) 4.27 (d, J = 19.96 Hz, 1 H) 3.01 (dd, J = 17.16, 6.06 Hz, 1 H) 2.76 (d, J = 17.18 Hz, 1 H) 1.14−1.16 (m, 9 H) 9.11 (s, 1 H) 6.82 Hz, 3 H). 4-Oxo-3,4,5,7-tetrahydro-pyrralo[3,4-d]pyrimidine-6-carboxylic Acid Tert-Butyl Ester (24). To a solution of 4-oxo-pyridine-1,3-dicarboxylic acid 1-tert-butyl ester 3-ethyl ester (46 g, 179 mmol) in EtOH (1.5 L), formamide hydrochloride (112 g 1073 mmol) was added, followed by NaOEt (203 g, 626 mmol). The reaction was heated at 90 °C for 2 h. The reaction mixture was then evaporated, and a saturated solution of ammonium chloride (300 mL) was added followed by DCM (1 L). The layers were separated, and the aqueous layer was extracted further with DCM. The organics were combined, dried, and evaporated to give the crude product. The title compound was purified using silica gel FCC (gradient elution 100% DCM to 94% DCM/6% MeOH) (10 g, 24% yield). 1H NMR (400 MHz, methanol-d) δ 8.18 (s, 1 H) 4.47−4.54 (m, 4 H) 1.45−1.56 (m, 8 H) 1.41 (s, 1 H) MS (ESI) m/z 238.2 (M + 1).
aqueous NH4Cl (1 L), the resulting slurry was then extracted with DCM (3 × 700 mL), and the combined organic layers were dried over sodium sulfate, filtered, and concentrated. The residue was separated directly via FCC (20–50% EtOAc/heptane) to give (S)-4-[(2-tert-butoxycarbonyl-5-[(1-methyl-cyclopentyl)-2H-pyrrozol-3-ylcarbamoyl]-1H-indol-5-yl oxy]-6-methyl-5,8-dihydro-6H-pyrido[3,4-d]pyrimidine-7-carboxylic acid tert-butyl ester (6i) (6.1 g, 9.4 mmol) in DCM (50 mL) was cooled to 0 °C. The residue was taken up in water (250 mL) and acetonitrile (25 mL) and stirred for 15 min before the solid was collected and filtered. The solution was filtered and then dried to give the title compound 33a (3.87 g, 92%). MS (ESI) m/z 444.1 (M + H)+. 1H NMR (400 MHz, DMSO-d6) δ 13.13 (brs, 1 H), 10.55 (m, 1 H), 8.40 (s, 1 H), 8.29 (d, J = 8.8 Hz, 1 H), 8.16 (d, J = 3.5 Hz, 1 H), 7.40 (d, J = 2.3 Hz, 1 H), 7.08 (d, J = 9.1 Hz, 2 H), 6.70 (d, J = 3.8 Hz, 1 H), 6.30 (s, 1 H), 3.76–3.96 (m, 2 H), 2.92–3.05 (m, 1 H), 2.85 (dd, J = 16.5, 3.4 Hz, 1 H), 2.27–2.40 (m, 1 H), 1.41 (s, 3 H), 1.22 (d, J = 6.3 Hz, 3 H), 0.89–0.97 (m, 2 H), 0.73–0.82 (m, 2 H).

5-(6-Hydroxymethyl-pyrimidin-4-yl)-indole-1-carboxylic acid (3-Trifluoromethylphenyl)-amide (5a). MS (ESI) m/z 429.1 (M + 1)+. 1H NMR (400 MHz, methanol-d4) δ 8.51 (s, 1 H), 8.37 (d, J = 8.84 Hz, 1 H), 8.07 (s, 1 H), 7.86 (s, 1 H), 7.79 (dd, J = 7.96, 1.39 Hz, 1 H), 7.58 (t, J = 8.97 Hz, 1 H), 7.45 (s, 1 H), 7.43 (d, J = 2.27 Hz, 1 H), 7.13 (dd, J = 8.97, 2.40 Hz, 1 H), 7.08 (d, J = 1.01 Hz, 1 H), 6.76 (d, J = 3.79 Hz, 1 H), 4.63 (s, 2 H).

N-(3-(8-fluoro-1,3,4,5-tetrahydro-1H-pyrido[3,4-d]pyrimidine-7-yl)-1H-indole-1-carboxamide (6a). MS (ESI) m/z 442.1 (M + 1)+. 1H NMR (400 MHz, methanol-d4) δ 8.64 (s, 1 H), 8.34 (d, J = 9.09 Hz, 1 H), 8.05 (s, 1 H), 7.93 (d, J = 3.79 Hz, 1 H), 7.89 (d, J = 8.08 Hz, 1 H), 7.56 (t, J = 7.96 Hz, 1 H), 7.40–7.46 (m, 2 H), 7.10 (d, J = 8.97, 2.40 Hz, 1 H), 6.99 (s, 1 H), 6.73 (d, J = 3.79 Hz, 1 H), 3.77 (s, 2 H), 2.40 (s, 3 H).

N-(4-Fluoro-3-(trifluoromethyl)phenyl)-5-[(6-[[((methylamino)methyl]pyrimidin-4-yl)-1H-indole-1-carboxamide (6b). MS (ESI) m/z 460.1 (M + 1)+. 1H NMR (400 MHz, methanol-d4) δ 8.65 (d, J = 1.01 Hz, 1 H), 8.35 (d, J = 8.84 Hz, 1 H), 8.04 (dd, J = 6.19, 2.65 Hz, 1 H), 7.90–7.94 (m, 2 H), 7.41 (d, J = 2.27 Hz, 1 H), 7.35 (t, J = 9.60 Hz, 1 H), 7.11 (dd, J = 8.97, 2.40 Hz, 1 H), 7.00 (s, 1 H), 6.74 (d, J = 3.79 Hz, 1 H), 3.82 (s, 2 H), 2.43 (s, 3 H).

5-[(5,6,7,8-tetrahydro-4-(4,3-diaminopyridin-4-yl)-indole-1-carboxamide (4a). MS (ESI) m/z 454.0 (M + 1)+. 1H NMR (400 MHz, DMSO-d6) δ 10.43 (s, 1 H), 8.54 (s, 1 H), 8.30 (d, J = 8.8 Hz, 1 H), 8.15 (d, J = 3.5 Hz, 1 H), 7.81 (s, 1 H), 7.98 (d, J = 8.3 Hz, 1 H), 7.65 (t, J = 8.0 Hz, 1 H), 7.44–7.53 (m, 2 H), 7.14 (d, J = 9.0, 2.4 Hz, 1 H), 6.81 (d, J = 3.8 Hz, 1 H), 4.26 (s, 2 H), 3.45 (t, J = 5.9 Hz, 2 H), 3.00 (t, J = 5.8 Hz, 2 H).

5-[(5,6,7,8-tetrahydro-4-(4,3-diaminopyridin-4-yl)-indole-1-carboxamide (3a). MS (ESI) m/z 482.2 (M + 1)+. 1H NMR (400 MHz, methanol-d4) δ 8.47 (d, J = 1.77 Hz, 1 H) 8.28 (dd, J = 9.09, 1.77 Hz, 1 H) 8.02 (s, 1 H) 7.85 (d, J = 3.66, 2.40 Hz, 2 H) 7.49 (t, J = 7.83 Hz, 1 H) 7.36 (d, J = 2.02 Hz, 1 H) 7.38 (d, J = 8.59 Hz, 1 H) 7.06 (d, J = 8.84, 2.27 Hz, 1 H) 6.65 (d, J = 2.53 Hz, 1 H) 4.63 (d, J = 6.06 Hz, 1 H) 4.15–4.23 (m, 1 H) 4.05–4.12 (m, 1 H) 1.55 (d, J = 6.57 Hz, 3 H).

R-5-(Methyl-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidine-4-carboxylic acid (3-N-(3-(trifluoromethyl)phenyl)-1H-indole-1-carboxamide (33a). MS (ESI) m/z 454.9 (M + 1)+. 1H NMR (400 MHz, methanol-d4) δ 8.47 (d, J = 1.77 Hz, 1 H) 8.28 (dd, J = 9.09, 1.77 Hz, 1 H) 8.02 (s, 1 H) 7.85 (d, J = 3.66, 2.40 Hz, 2 H) 7.49 (t, J = 7.83 Hz, 1 H) 7.36 (d, J = 2.02 Hz, 1 H) 7.38 (d, J = 8.59 Hz, 1 H) 7.06 (d, J = 8.84, 2.27 Hz, 1 H) 6.65 (d, J = 2.53 Hz, 1 H) 4.63 (d, J = 6.06 Hz, 1 H) 4.15–4.23 (m, 1 H) 4.05–4.12 (m, 1 H) 1.55 (d, J = 6.57 Hz, 3 H).
5-(6,7,8-Tetrahydro-pyrido[3,4-d]pyrimidin-4-yl)-indole-1-carboxylic Acid (5-tert-Butyl-2H-pyrrol-3-yl)-amide (33m). MS (ESI) m/z 432.1 (M + 1); 1H NMR (methanol-d4) δ 8.37 (s, 1H), 8.32 (d, J = 8.8 Hz, 1H), 7.78 (d, J = 3.5 Hz, 1H), 7.74 (d, J = 2.3 Hz, 1H), 7.08 (dd, J = 9.1, 2.3 Hz, 1H), 6.71 (d, J = 3.8 Hz, 1H), 6.37 (s, 1H), 3.84 (s, 2H), 3.19 (t, J = 5.9 Hz, 2H), 2.89 (t, J = 5.7 Hz, 2H), 1.36 (s, 9H).

5-(6,7,8-Tetrahydro-pyrido[3,4-d]pyrimidin-4-yl)-indole-1-carboxylic Acid [5-(1-Methyl-cyclopropyl)-2H-pyrrol-3-yl]-amide (33n). HRMS (ESI+) m/z calculated for C21H18N6O3 (M + 1) 403.1520; 1H NMR (400 MHz, DMSO-d6) δ 8.56 (s, 1H), 8.29 (d, J = 8.84 Hz, 1H), 8.17 (d, J = 3.79 Hz, 1H), 7.46 (d, J = 2.27 Hz, 1H) 7.14 (d, J = 8.97, 2.40 Hz, 1H) 6.75 (d, J = 3.79 Hz, 1H) 6.68 (s, 1H) 4.62 (d, J = 6.57 Hz, 1H) 4.12 (d, J = 2.27 Hz, 1H) 4.08 (d, J = 1.52 Hz, 1H) 1.44 (d, J = 6.57 Hz, 3H) 1.34 (s, 9H).

5-(6,7-Dihydro-5H-pyrrrole[3,4-d]-pyrimidin-4-yl)-indole-1-carboxylic Acid (5-Cyclopentyl-isoxazol-3-yl)-amide (33o). HRMS (ESI+) m/z calculated for C21H18N6O3 (M + 1) 417.1415; 1H NMR (400 MHz, DMSO-d6) δ 8.56 (s, 1H), 8.29 (d, J = 8.84 Hz, 1H), 8.17 (d, J = 3.79 Hz, 1H), 7.47 (d, J = 2.27 Hz, 1H) 7.15 (dd, J = 8.97, 2.40 Hz, 1H) 6.75 (d, J = 3.79 Hz, 1H) 6.67 (d, J = 8.54–8.6 Hz, 1H) 6.04–6.16 (m, 4H) 2.07–2.26 (m, 1H) 1.01–1.15 (m, 2H).

Journal of Medicinal Chemistry

We used female C57BL6 mice that were typically 8–10 weeks old. Mice used in an experiment arrived at our research facility in a single shipment. Experimental interventions for a cohort of animals were started on the same day. Mice were treated by oral gavage with compound starting 1 h before laser treatment (Day 0) and continuing once a day through Day 6 after laser treatment. Experimental groups were dosed with either vehicle (0.5% methylcellulose and 0.2% Tween 80) or compound in vehicle (0.5% methylcellulose and 0.2% Tween 80) at 10 mg/kg. Each group consisted of 10 mice, with 3 laser burns to each eye yielding 60 data per group.

Laser Photocoagulation. Mouse pupils were dilated with one drop (10% v/v) of 1% cyclopentolate. Just before anesthesia, pupil dilation was maximized with an additional drop of phenylephrine (usually 10% but occasionally 2.5% depending upon availability). Mice were then anaesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine and xylazine at doses up to 100 mg/kg and 10 mg/kg, respectively. Prior to laser pulse application, each mouse was anaesthetized with topical 0.5% proparacaine. Lubricating eye drops (Genteal Novaris) on a glass coverslip was applied to the cornea, and the retina was viewed through a slit lamp microscope. Three laser pulses were applied to the fundus around the optic nerve of each eye, six laser pulses in total for each mouse. The pulses were from a green laser (wavelength = 532 nm; Oculight GLx) and had a duration of 30 ns, a power of 120 mW, and a spot size of 100 μm. A successful laser burn generated a yellow vaporization bubble which correlated with a rupture of Bruch’s membrane. If a vaporization bubble did not form, one additional laser pulse would be administered to the same spot. For each eye, a maximum of four shots were allowed. An additional fourth shot was required in fewer than 3% of eyes. After the application of laser burns to both eyes, antibiotic ointment (0.5% erythromycin, Tobramycin, or Neomycin ophthalmic ointment depending on availability and cost) was applied to both eyes.

Harvest and Photography of CNV Lesions. Unless indicated, studies were completed 7 days after laser photocoagulation. On the
final study day, 0.1 mL of a 5 mg/mL solution of FITC concanavalin-A (Vector Laboratories) was injected intravenously (i.v.) to fluorescently label the vascular endothelium. Animals were sacrificed 15–30 min later with inhaled carbon dioxide. Eyes were enucleated and fixed in 4% paraformaldehyde for approximately 60 min at room temperature and then transferred to vials of PBS. Posterior eye cups were dissected from the eyes. The RPE-choroidal-sclera complexes were separated from the neural retina and mounted on microscope slides, at which time each eye was given a randomization code to mask the examiners. Fluorescent images of each CNV lesion were photographed with an AxioCam MR3 camera on an Axio Image M1 microscope (Zeiss). CNV area was quantified using a semiautomated analysis program (Axiovision software, version 4.5, Zeiss) that outlined the fluorescent blood vessels. Image capture, CNV area measurement, and exclusions (see below) were performed on masked microscope slides.

**Measurements of Neovascular Lesions.** Most eyes generated three data points corresponding to the area of each of the three laser-induced CNV lesions. A cohort of 10 mice with both eyes treated would optimally provide 60 data points. However, a lesion would be necessarily excluded for any of the following reasons: (1) there was choroidal hemorrhage encroaching on the lesion; (2) the lesion was of irregular shape indicating an asymmetrical laser burn; (3) the lesion had fused with another lesion; (4) the lesion had a size indicating that it was an outlier lesion, as defined below; or (5) the lesion was the only lesion in an eye (i.e., if 2 of the 3 lesions in an eye were excluded, then all lesions in that eye were excluded).

An outlier lesion fell into one of the following three categories: (1) "too big," i.e., it was over 10,000 μ² in area, and it was more than 5 times larger than the next biggest lesions in the eye (for reference, the mean area of CNV in a control group typically ranged from 10,000 to 20,000 μ²); (2) "too small," i.e., it was less than 1/5 the area of the other next smallest lesion in the eye; this criterion applied only if at least one lesion in the eye was over 5,000 μ²; (3) "too different," i.e., after all of the lesions in a specific treatment group were measured, the lesion’s area was 5-fold greater than the mean for that group; this criterion applied only for lesions that were ≥5,000 μ².

Other reasons that lesions were excluded or not measured included: (1) the death of a mouse before the end of an experiment; (2) the failure of the i.v. injection of the vascular label; (3) the lesion could not be applied due to media opacities (e.g., a pre-existing corneal scar or cataract); in this case, the fellow eye could still be included; (4) there was damage to the CNV lesion during tissue processing (if any part of the CNV was cut or not discernible, it was not included); and (5) a CNV lesion could not be located during the imaging of an eye cup.

**Laser-Induced Choroidal Neovascularization (CNV) Model.** We used male Brown Norway rats that were typically 12 weeks old (range of age 7–14 weeks). Rats were purchased from Harlan, and the rats used in a single experiment arrived at our research facility in a single shipment and were age-matched. Experimental interventions for a cohort of animals were started on the same day. Laser application for an individual experiment was by a single scientist. Cages were randomly assigned to treatment groups with rats sharing a cage receiving the same treatment.

**Laser Photocoagulation.** Rat pupils were dilated with one drop (volume ~40 μL) of 1% cyclopentolate. Just before anesthesia, pupil dilation was maximized with an additional drop of phenylephrine (usually 10% but occasionally 2.5% depending upon availability). Rats were then anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine and xylazine at doses of 40–80 mg/kg and 5–10 mg/kg, respectively. Prior to laser pulse application, each eye was anesthetized with topical 0.5% proparacaine. Lubricating eyedrops (Gentec Alcon Laboratories, Fort Worth, TX) on a glass coverslip were applied to the cornea, and the retina was viewed through a slit lamp microscope. Each laser pulse was applied approximately 1 mm from the optic nerve; single pulses in each of four separate locations were applied to each eye for a total of eight laser photocoagulation sites for each mouse. The pulses were from a green laser (wavelength = 532 nm; Oculight GLX Mountain View, CA) and had a duration of 30 μs, a power of 190 mW, and a spot size of 200 μm. A successful laser pulse generated a yellow vaporization bubble which correlated with a rupture of Bruch’s membrane (evaluated historically in control rats sacrificed 6 h after laser; data not shown). In cases when a vaporization bubble did not form (<1% of laser pulses), one additional laser pulse could be administered to the same spot. For each eye, a maximum of five laser pulses were allowed to generate 4 lesions. After the application of laser burns to both eyes, antibiotic ointment (Tobramycin with Neomycin ophthalmic ointment depending on availability and cost) was applied to both eyes.

**Tissue Processing, Imaging, and CNV Area Quantification.** Analysis of neovascularization was completed on tissues harvested 11, 12, or 14 days after laser photocoagulation depending on the dosing paradigm of the experiment. For an individual experiment, all rats were euthanized on the same day after laser photocoagulation. On the final study day, 0.2 mL of a 12.5 mg/mL solution of a 2,000 kDa FITC dextran (Sigma) was injected intravenously (i.v.) to fluorescently label the vascular endothelium. Animals were euthanized 15–30 min later with inhaled carbon dioxide. Eyes were enucleated and fixed in 4% paraformaldehyde (Vector Laboratories, Burlingame, CA) for approximately 60 min at room temperature, and then, the fixative was replaced with PBS. Each eye was assigned a randomized number to mask the samples for the remainder of the analysis. Posterior segments were isolated, and retinas were removed. The posterior eye cups which included the retinal pigment epithelium (RPE), the choroid, and the sclera were flat-mounted onto microscope slides after making 3 or 4 radial cuts. Fluorescent images of each CNV lesion were photographed with an AxioCam MR3 camera on an Axio Image M1 microscope (Carl Zeiss Microscopy, Thornwood, NY). CNV area was quantified using a semiautomated analysis program (Axiovision software, version 4.5, Carl Zeiss Microscopy) that outlined the fluorescent blood vessels. Image capture, CNV area measurement, and exclusions (see below) were performed on randomized samples or data by scientists masked to the treatment group.

**Compound Administration.** Test articles were administered via oral gavage, starting approximately 1 h before laser, unless described otherwise.

**Application of Exclusion Criteria.** Each eye typically generates 4 data points corresponding to the area of 4 individual CNV lesions. In a typical study, a cohort of 10 rats per group would optimally provide 80 data points. However, a lesion would be excluded for any of the following reasons: (1) there was choroidal hemorrhage encroaching on the lesion; (2) the lesion was linear instead of circular, a consequence of a deflected ("split") laser impact; (3) the lesion had fused with another lesion; (4) the lesion had a size indicating that it was an outlier lesion as defined below; or (5) the lesion was the only lesion in an eye (i.e., if 3 of the 4 lesions in an eye were excluded, then all lesions in that eye were excluded).

An outlier lesion fell into one of the following three categories: (1) "too big," i.e., it was over 10,000 μ² in area and was more than 5 times larger than the next biggest lesion in the eye (for reference, the mean area of CNV in a control group typically ranged from 10,000 to 20,000 μ²); (2) "too small," i.e., it was less than 1/5 the area of the other next smallest lesion in the eye; this criterion applied only for lesions that were ≥5,000 μ².

Other reasons that lesions were excluded or not measured included: (1) the death of a rat before the end of an experiment; (2) the failure of the i.v. injection of the vascular label; (3) media opacities precluding accurate laser application (e.g., a pre-existing corneal scar or cataract); in this case, the fellow eye could still be included; (4) there was damage to the CNV lesion during tissue processing (if any part of the CNV was cut or not discernible, it was not included); and (5) a CNV lesion could not be located during the imaging of an eye cup.
retina and the posterior eye cup. Each time point had drug levels measured in 4 individual retinas, 4 individual posterior eye cups, and 2 individual plasma samples.

Ocular tissues were homogenized and plasma proteins precipitated, and the drug concentration was analyzed by LC-MS/MS. Exposures in the retina, posterior eye cup (PEC), and plasma for the compounds and the drug concentration was analyzed by LC-MS/MS. Exposures in the retina, posterior eye cup (PEC), and plasma for the compounds and the drug concentration were dose normalized and are listed in Table 6 as area under the curve measurements (AUC), $C_{max}$, and $C_{\infty \text{ hr}}$.

**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: erik.meredith@novartis.com.*

**Present Addresses**

1. N.M.: Raze Therapeutics, 400 Technology Square, Cambridge, MA 02139.
2. J.K.: Department of Ophthalmology and Vision Science, University of Toronto, and Department of Vision Sciences, Toronto Western Research Institute, 399 Bathurst Street, Toronto, Ontario M5T 1K2, Canada.
3. L.J.: Coditas Group, 125 Westbourne Terrace, Brookline, MA 02446.
4. G.A.: Kalexsyn, 4502 Campus Drive, Kalamazoo, MI, 49008.
5. L.H.: Sensirion, Laubisruettistrasse, 508712 Staefa ZH, Switzerland.
7. C.R.: School of Biological Science, Nanyang Technological University, 60 Nanyang Drive, Singapore, 637551.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We acknowledge the contribution of the Novartis Institute for Biomedical Research Analytical Sciences group and the Novartis Institute for Biomedical Research Metabolism and Pharmacokinetics group for support in generating the analytical details for the compounds described herein.

**ABBREVIATIONS USED**

TLC, thin layer chromatography; VEGF, vascular endothelial growth factor; AMD, age-related macular degeneration; ADME, absorption distribution metabolism excretion; CNV, choroidal neovascularization; PEC, posterior eye cup; p.o., oral; i.v., intravenous; i.v.t., intravitreal; ETDRS, Early Treatment of Diabetic Retinopathy Study; WFI, water for injection; MC, methylcellulose; CMC, carboxymethylcellulose; DN, dose normalized

**REFERENCES**


(21) Bold, G.; VaupeL, A.; Lang, M. Heterobicyclic Carboxamides as Protein Kinase Inhibitors and Their Preparation, Pharmaceutical


