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Design and structure–activity relationship of heterocyclic analogs of 4-amino-3-benzimidazol-2-ylhydroquinolin-2-ones as inhibitors of receptor tyrosine kinases

Kelly Frazier, Elisa Jazan, Christopher M. McBride, Sabina Pecchi, Paul A. Renhowe, Cynthia M. Shafer,* Clarke Taylor, Dirksen Bussiere, Molly Min He, Johanna M. Jansen, Gena Lapointe, Sylvia Ma, Jayesh Vora and Marion Wiesmann

Small Molecule Drug Discovery, Biopharma Division, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608, USA

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Abstract—Herein are described a series of novel heterocyclic analogs of the 4-amino-3-benzimidazol-2-ylhydroquinolin-2-one scaffold. These compounds are potent inhibitors of receptor tyrosine kinases and exhibit favorable pharmacokinetic profiles. The synthesis and SAR of these compounds are described.

Receptor tyrosine kinases (RTKs) and their ligands are involved in important signal transduction pathways within the cell. In particular, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), and their receptors play fundamental roles in cell proliferation, differentiation, motility, and apoptosis.[1–4] Under aberrant growth conditions as in cancer, these signal transduction pathways can be deregulated and contribute to sustained angiogenesis and evasion of apoptosis of tumor cells.[5,6] Inhibition of receptor tyrosine kinases represents an attractive therapeutic modality in oncology, and a number of RTK inhibitors are currently being evaluated preclinically or in clinical trials.[7–9]

3-Benzimidazol-2-ylhydroquinolin-2-ones (Fig. 1) are a class of potent VEGFR, FGFR, and PDGFR RTK family inhibitors with attractive physicochemical and pharmacokinetic properties and significant efficacy in murine and human xenograft tumor models.[10–12] Concurrent with the development of the SAR around the 3-benzimidazol-2-ylhydroquinolin-2-one series, we explored several other classes of compounds where the A or D aryl ring (Fig. 1) was replaced with a five- or six-membered ring heterocycle.

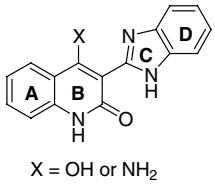

Figure 1. The carbocyclic 3-benzimidazol-2-ylhydroquinolin-2-one scaffold.

Ring A and ring D pyridino-analogs were synthesized in a manner analogous to the carbocyclic series[13] using a LHMDS promoted one-pot tandem acylation–cyclization (Scheme 1). The desired heteroaromatic aminonitriles[14–20] and azabenzimidazole acetates[21,22] were synthesized as described in the literature or with slightly modified procedures.

Table 1 shows a comparison between compounds in the carbocyclic series (compounds **1**–**4**) and the A ring pyridyl series (compounds **5**–**11**). The 3-benzimidazol-2-yl-4-hydroxyhydroquinolin-2-one analog (**1**) was found to be 6-fold less potent against VEGFR-1 than the corresponding 4-amino analog (**2**). An additional potency improvement was obtained with the introduction of a basic amine on ring D (**3** and **4**).

Keywords: Vascular endothelial growth factor (VEGF); Basic fibroblast growth factor (bFGF); Platelet derived growth factor (PDGF).
* Corresponding author. Tel.: +1 510 923 8086; e-mail: cynthia_shafer@chiron.com

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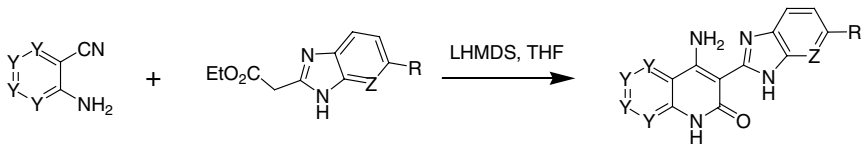
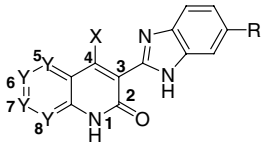
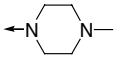
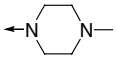
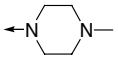


Scheme 1. Synthetic scheme for synthesis of heterocyclic analogs of 4-amino-3-benzimidazol-2-ylhydroquinolin-2-ones.

Table 1. SAR comparing the carbocyclic series with the ring A heterocyclic analogs



Compound	X	Y	R	VEGFR-1 IC_{50} (μM)	*m*-VEGFR-2[a] IC_{50} (μM)	*m*-PDGFRβ[a] IC_{50} (μM)	FGFR-1 IC_{50} (μM)	HMVEC EC_{50} (μM)
1	OH	CH	H	0.162	0.114	0.395	0.215	0.428
2	NH_2	CH	H	0.028	0.025	0.102	0.044	0.078
3	NH_2	CH		0.012	0.004	0.038	0.021	0.040
4	NH_2	CH		0.010	0.013	0.027	0.008	0.020
5	OH	8-N	H	3.94	3.00	6.53	5.00	>10
6	OH	7-N	H	0.184	0.168	1.59	0.576	2.20
7	NH_2	7-N	H	0.004	0.003	0.057	0.010	0.053
8	NH_2	7-N		0.004	0.002	0.064	0.010	0.084
9	NH_2	7-N		0.003	0.001	0.043	0.004	0.016
10	NH_2	6-N		0.037	0.057	0.018	0.100	0.768
11	NH_2	5-N		0.074	0.033	0.055	0.043	0.089

[a] For both of the biochemical VEGFR2 and PDGFRβ assays, the highly conserved mouse homologs have been used.

The 3-benzimidazol-2-ylhydropyridino pyridin-2-one series (Scheme 1 in which one of the Y = N) demonstrates a marked dependence of the activity on the position of the nitrogen within the ring. Replacing C-8 with a nitrogen led to >20-fold loss of activity (Table 1, **5** vs **1**). The same replacement at C-7, however, resulted in a compound (**6**) inhibiting VEGFR-1 with a potency comparable to that of the carbocyclic analog (**1**). Furthermore, changing the substituent at C-4 from hydroxyl to amino (**7**) greatly improved potency in both the biochemical and cell based assays measuring VEGF-mediated proliferation in endothelial cells. Incorporating a basic amine on the D ring gave similarly potent biochemical results with improved physicochemical properties (**8** and **9**).[23] Moving the nitrogen to the C-6 position (**10**) led to a 3- to 9-fold decrease in potency against VEGFR-1 compared to the carbocyclic analog (**3**) and the 7-N pyridyl (**8**), respectively. The slight increase in affinity for PDGFRβ and decrease for FGFR-1 suggests N-6 as a potential handle for

PDGFRβ selective compounds. However, compound **10**'s cellular activity is 19-fold worse than the carbocyclic analog (**3**) and 9-fold worse than the least potent heterocyclic analog. Last, incorporation of the nitrogen at the 5 position of ring A (**11**) resulted in a 7-fold loss of enzymatic activity against VEGFR-1 and a similar loss of cellular potency compared to the carbocycle (**4**). It is worth noting that, irrespective of the structural modification, the activity against the two isoforms of the VEGF receptor is always very similar in both the carbocyclic and heterocyclic series.

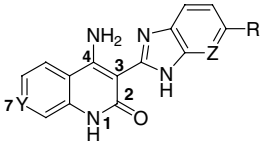
The effect of incorporating a nitrogen into the D ring was then evaluated. The 4-amino-3-imidazolo[4,5-*b*]pyridin-2-ylhydroquinolin-2-one series (Scheme 1, Z = N) shows a structure–activity relationship nearly identical to that of the carbocyclic series (Table 2). Combination of the most potent A ring heterocyclic series (7-N) with the azabenzimidazole series (**13**) gave IC_{50} values consistent with ring A heterocycle replacement alone. Besides offering no advantage with respect to in vitro biochemical and cellular activity, the azabenzimidazole analogs also exhibited inferior aqueous solubility.[24]

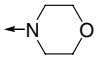
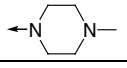
One additional structural modification to be carried out was replacement of the six-membered ring A with a five-membered ring heterocycle, such as thiophene, imidazole or pyrazole. A two-step synthesis (Scheme 2) was, in some cases (i.e., **18**), necessary for this series because the amide intermediate would not cyclize under the LHMDS/THF conditions. In those cases, the amide, which could be identified by the diagnostic ^{1}H NMR signal of the α methylene,[25] was isolated and treated with NaOMe in MeOH at 90 °C overnight, to yield the desired cyclized product (as confirmed by ^{1}H NMR) in low yield (<10%). Attempts to incorporate a morpholino group on ring D of **16** to potentially improve in vitro potencies failed, as decomposition occurred when the amide was subjected to the NaOMe/MeOH conditions. The activities of these compounds in the biochemical assays are reported in Table 3. In general, the affinities for all targets are lower for the five-membered ring heterocycles compared to the heterocyclic six-membered A-rings. The thiophene (**16**) was the most potent of these analogs with an IC_{50} of 0.132 μM against VEGFR-1. Compound **16** also showed modest selectivity against PDGFRβ, while the imidazole (**17**) and pyrazole (**18** and **19**) analogs were significantly less potent against FGFR-1. In addition, compounds **17** and **19** demonstrated a 4- to 8-fold selectivity for PDGFRβ versus VEGFR-1, with compound **19**, where the pyrazole nitrogen is methylated, exhibiting the best selectivity.

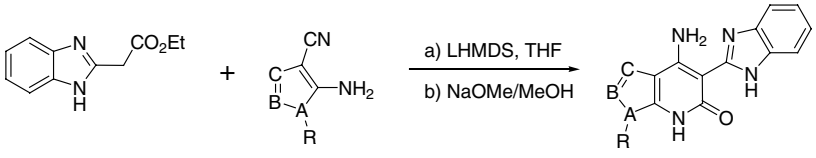
Pharmacokinetic experiments in mice (Table 4) showed that ring A (**8**) and ring D (**14**) pyridyl analogs both have high oral bioavailability on par with the carbocyclic parent (**3**). Compound **8** had a moderate clearance but a very short iv half-life. Compound **14** had an iv half-life similar to the carbocyclic parent (**3**) but higher hepatic clearance.

In order to understand interactions of these compounds with the ATP binding site of VEGFR-2, a docking model of compound **8** in the active site of a homology

Table 2. SAR comparing the carbocyclic parent with the ring D heterocyclic analogs



Compound	Y	Z	R	VEGFR-1 IC_{50} (μM)	*m*-VEGFR-2 IC_{50} (μM)	*m*-PDGFRβ IC_{50} (μM)	FGFR-1 IC_{50} (μM)	HMVEC EC_{50} (μM)
12	CH	N	H	0.022	0.016	0.025	0.051	0.088
13	7-N	N	H	0.006	0.012	0.049	0.030	0.077
14	CH	N		0.010	0.008	0.048	0.047	0.034
15	CH	N		0.014	0.024	0.030	0.010	0.051



Scheme 2. Synthetic scheme for five-membered ring A analogs.

Table 3. SAR of five-membered ring A analogs

Compound	A	B	C	R	VEGFR-1 IC_{50} (μM)	*m*-VEGFR-2 IC_{50} (μM)	*m*-PDGFRβ IC_{50} (μM)	FGFR-1 IC_{50} (μM)	HMVEC EC_{50} (μM)
16	S	CH	CH	—	0.132	0.109	0.691	0.348	0.850
17	N	CH	N	H	0.602	0.552	0.154	2.66	3.68
18	N	N	CH	H	0.691	0.507	0.326	3.17	3.82
19	N	N	CH	Me	4.30	3.00	0.575	3.00	0.321

Table 4. Pharmacokinetic profile comparison of carbocyclic and ring A heterocycle analogs (iv 5 mg/kg, po 20 mg/kg)

Compound	*F* (%)	CL (mL/min/kg)	$t_{1/2}$ (iv, min)	$t_{1/2}$ (po, min)
3	100	13	71	216
8	87	24	26	78
14	84	61	65	57

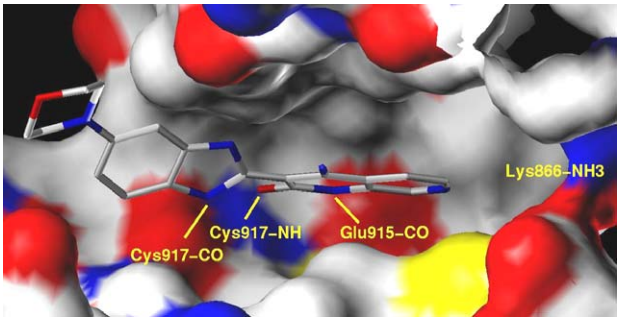


Figure 2. Docking model of compound **8** in VEGFR-2 homology model. Surface of the binding site is colored by atom-type (oxygen = red, nitrogen = blue, and carbon = white). Hydrogen bonds to hinge residues are labeled, as is the surface from the catalytic Lys866.

model of VEGFR-2 was developed.[26] In this model, the core of compound **8** forms three hydrogen bonds to the hinge domain of the kinase as indicated in Figure 2, while the morpholino group points out into solvent. The slight increase in affinity as observed for the 7-position pyridyl analogs could be due to an interaction with the catalytic Lys866. This residue is known to be flexible and could reach the 7-position for hydrogen bonding. The loss of affinity for the N-8 analog, compound **5**, could be due to unfavorable interactions between the pyridyl-N and the backbone Glu915-CO.

In conclusion, replacement of ring A with a five-membered ring heterocycle (thiophene, imidazole or pyrazole) resulted in analogs with reduced affinity against kinases involved with angiogenesis and blood vessel maintenance. The in vitro potency of ring D pyridyl analogs, on the other hand, was quite similar to that of the carbocyclic series. The disadvantage of the ring D heterocycles, however, was both the inferior solubility and the high hepatic clearance. Ring A six-membered heterocyclic analogs varied in their potency, with the N-7 analogs being slightly more potent than the parent carbocycle. This series, however, had higher hepatic clearances and shorter in vivo half-lives, which made them unsuitable for further development.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.020.

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23. Compared to **7**, compounds **8** and **9** showed a 4-fold and >50-fold solubility improvement, respectively.
24. Unpublished result.
25. Uncyclized amide intermediate can be distinguished by a diagnostic signal of the methylene protons at 4.0–4.3 ppm.
26. The VEGFR-2 homology model was built using Chemical Computing Group's MOE software. Default settings were used in the alignment and homology modeling module and the FGFR1 crystal structure (2FGI) from the Brookhaven Protein Data Bank was used as a template. Compound **8** was built and optimized in the active site of the homology model using the Flo+apr2003 software from Thistlesoft.