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SAR of a novel 'Anthranilamide Like' series of VEGFR-2, multi protein kinase inhibitors for the treatment of cancer

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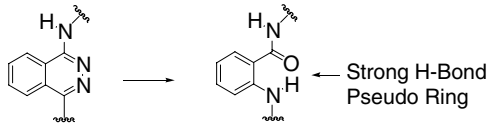
Abstract—Novel anthranilamides were surprisingly found to exert additional activity on B-RAF. Corresponding thiophene, pyrazole, and thiazole core analogs were prepared as VEGFR-2 inhibitors with c-KIT, and B-RAF activity. Compounds in the phenyl, thiophene, and thiazole series are in vivo active.

Cancerous tumor growth depends on the formation of blood vessels for oxygen and nutrients supply. Initiation of blood vessel formation and growth is mediated through pro-angiogenic growth factor receptors such as VEGFR-2 (Vascular endothelial growth factor receptor-2). Inhibitors of this growth factor receptor have been postulated to reduce tumor size by reducing blood vessel growth and thus starving the tumor of oxygen and nutrients. As a result, VEGFR-2 inhibition has become a major area of interest in experimental and approved tumor therapy.[1–3] This can be exemplified by the great deal of attention that PTK787 (vatalanib), one of the most advanced VEGFR-2 inhibitors, has attracted.[4–6]

More recently, it has been shown that substituted anthranilamides can act as aminophthalazine mimics leading to potent VEGFR-2 inhibitors (Scheme 1).[7]

Based on a continued interest in VEGFR-2 inhibitors and their activity profiles, a chemical library of anthranilamides was synthesized, and broadly screened against a panel of tumor associated kinases. In addition to the expected VEGFR-2 activity, compounds with c-KIT (a cell surface, growth[8] and survival[9] promoting, receptor tyrosine kinase) and B-RAF (a serine/threonine kinase receptor associated with cell proliferation and survival of which mutations are often found in cancer[10]) activity were discovered, leading to a program aimed at optimization of these activities in parallel.[11,12]

Variation of the initial screening hit, anthranilamide **1**, rapidly led to pyridyl amide **2** as the lead structure. This



Scheme 1.

Keywords: Kinase; Cancer; Anthranilamide.
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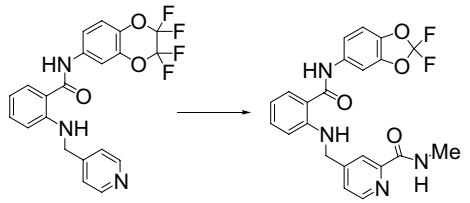
compound possessed in vivo activity in three different human xenograft models (data not shown) and served as the starting point for the program in which the different regions of the lead were subjected to systematic structural variation.



Screening Hit **1**
c-Kit IC_{50} =19 nM
B-RAF IC_{50}=40 nM
PK AUC = 6 μM*h

Lead **2**
c-Kit IC_{50} =5 nM
B-RAF IC_{50}=45 nM
PK AUC = 40 μM*h

(PK - Female ICR mice, 25 mg/kg, PO)

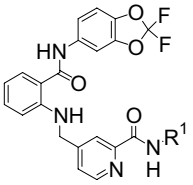
From comparison between analogs of the initial library it was deduced, that the activity against B-RAF is inherent to the fluorinated aniline part of **1** and **2**.

Exploration of the hinge binding motif that would maintain the biological activities identified in the lead, and possibly build on them, was next undertaken. It is generally assumed that the pyridyl moiety is required, as it has been shown to occupy the ATP adenine binding pocket and form a hydrogen bond to the hinge region.[13] The methyl amide moiety further strengthens the interaction by formation of a second hydrogen bond as has been demonstrated by X-ray crystallography of the BAY 43-9006 (sorafenib)/B-RAF complex.[14] Variations of the pyridylamide moiety and attachment of solubilizing chains were next explored (Table 1). The c-KIT and B-RAF activities were in general weaker than for the parent (Lead **2**). Particularly analogs with solubilizing chains showed weaker activity, especially with respect to B-RAF. The smaller the amide substitution, the more active the molecule is at both receptors.

A number of other known hinge binding motifs were studied (Table 2). Initially the most attractive motif was reversed amide **14**, as it had low single digit activity at both c-KIT and B-RAF. Unfortunately this compound was determined to be unstable in vivo. Methyl amino analog **19** was the most active compound in the pyrimidine series. Of the few compounds made in this series, the larger the substituent on the amine, the less active the molecules were. As the activity of this series was limited to substituents smaller than a methyl it was not explored further.

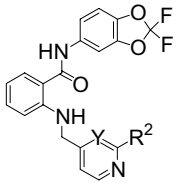
Pyridyl amines were examined as a way to combine the NH-function of the reverse amide **14** with the amino group of the pyrimidine **19** which should increase the stability of the compound. Indeed, compounds **24** and **27** had much increased stability which resulted in increased exposure in mice as compared to **14**. Unfortunately all the other compounds in the series generated much lower plasma levels. The B-RAF activity for these

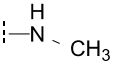
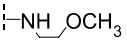
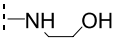
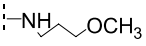
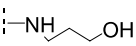
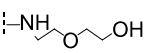
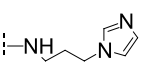
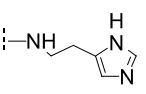
Table 1. Hinge binder SAR—pyridyl amide with solubilizing chains



Compound	R^1	c-KIT IC_{50} (nM)	B-RAF IC_{50} (nM)
3	H	2	34
4	CH_2CH_3	25	333
5	$CH_2CH_2N(Et)_2$	58	138
6	$CH_2CH_2CH_2N(Et)_2$	42	175
7	$(CH_2)_3$Morpholino	45	6830
8	Cyclopropyl	16	192
9	$CH_2CH_2SO_2CH_3$	17	221
10	$CH_2CH_2OCH_3$	38	497
11	$CH_2CH_2CH_2OCH_3$	19	1030
12	CH_2CH_2OH	18	397

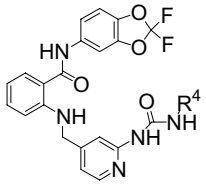
Table 2. Hinge binder SAR



Compound	Y	R^2	c-KIT IC_{50} (nM)	B-RAF IC_{50} (nM)
13	CH	NH_2	1	274
14	CH	$NHCOCH_3$	1	5
15	CH	CN	37	110
16	CH	$CSNH_2$	27	100
17	CH		72	153
18	CH	$NHS(O)_2CH_3$	25	594
19	N	$NHCH_3$	3	26
20	N	$N(CH_3)_2$	50	349
21	CH	NH_2	4	634
22	CH		3	100
23	CH		13	104
24	CH		8	112
25	CH		12	156
26	CH		9	142
27	CH		4	165
28	CH		20	101
29	CH		23	146

compounds was also weak and thus the lack of plasma exposure and weak B-RAF activity kept this series from advancing further.

Table 3. Hinge binder SAR—pyridyl ureas



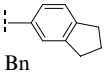
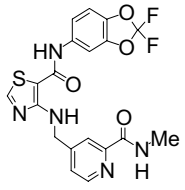
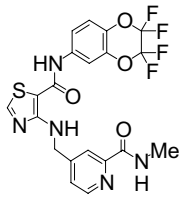
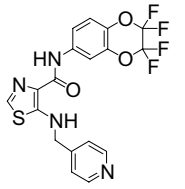
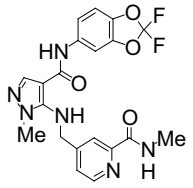
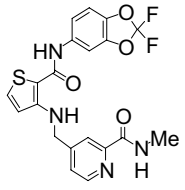
Compound	R^4	c-KIT IC_{50} (nM)	B-RAF IC_{50} (nM)
30	CH_3	5	24
31	CH_2CH_3	7	90
32	Ph	87	39
33		240	64
34	Bn	48	232

Table 4. Core SAR

Compound	Structure	c-KIT IC_{50} (nM)	B-RAF IC_{50} (nM)
35		25	1240
36		12	192
37		95	2560
38		11	199
39		3	61

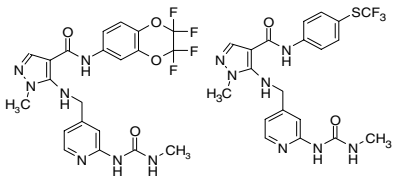
The final modification to the amino pyridine examined to increase the stability was capping the amine in the form of a urea (Table 3). In line with the findings discussed previously, the smaller substituents gave the more active compounds. Methyl urea **30** and ethyl urea **31** had promising biochemical activity and were highly exposed in mice (AUC_{0-5h} > 400 μM*h at 25 mg/kg po).

We next focused our attention on new cores replacing the phenyl of the anthranilamide by heterocycles. Of the cores studied, the thiazole (**36**), pyrazole (**38**), and thiophene (**39**) looked the most promising with regard to their activity (Table 4). The thiophene core (example **39**) has since been published.[15] The other two thiophene regioisomers were either less active or unstable.

Further core replacements such as *trans*-cyclohexane, cyclopentene, and *cis*-cyclopentane were all synthesized and had very little activity. Planar molecules seem to be more active in general.

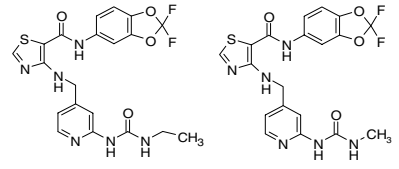
The new cores (pyrazoles and thiazoles) were then combined with some of the hinge binding motifs explored in the phenyl series. The most promising compounds resulting from these efforts are shown in Tables 5 and 6. Thiazoles (Table 6) tended to be slightly more active than

Table 5. Pyrazole core combined with pyridyl urea

Structure 

	40	**41**
c-KIT IC_{50} nM	12	17
P-AKT IC_{50} nM	41	15
B-RAF IC_{50} nM	191	154
P-ERK IC_{50} nM	448	899
PK 15 mpk C_{max} μM	6	3.6
AUC_{1-5h}μM*h	19	11
VEGFR-2 IC_{50} nM	43	62

Table 6. Thiazole core combined with pyridyl urea

Structure 

	42	**43**
c-KIT IC_{50} nM	4	5
P-AKT IC_{50} nM	81	24
B-RAF IC_{50} nM	122	69
P-ERK IC_{50} nM	824	508
PK 25 mpk C_{max} μM	64	124
AUC_{1-5h}μM*h	255	538
PK-IV V_{dss} (L/kg)	0.24	0.151
VEGFR-2 IC_{50} nM	7	3

pyrazoles. However in orienting PK studies in mice, thiazoles displayed a much higher exposure compared to pyrazoles and therefore the thiazole derivatives were selected for in-depth characterization.

Ethyl urea **42** and methyl urea **43** were both very active in the c-KIT assay and slightly less active in the B-RAF assays. Strong VEGFR-2 activity was also confirmed for these analogs (Table 6). Both compounds are active in vivo against three human tumor xenograft models (MDA-MB-231 breast, Colo 205 colon, NCI-H460 NSCLC) grown subcutaneously in athymic mice. The tumor growth inhibitions for **42** at 50 mg/kg po once daily given for 9 days are 92%, 86%, and 79%, respectively. The tumor growth inhibitions for **43** at 50 mg/kg are 90%, 84%, and 70%, respectively.

In conclusion, a series of novel anthranilamides and their corresponding heterocyclic analogs has been shown to possess potent c-KIT, B-RAF, and VEGFR-2 activity. Lead compounds in this series display potent in vivo activity. Further information on these compounds will be published in due course. Synthesis of these compounds can be found in the patent literature.[16–18]

Acknowledgments

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11. In general the c-Kit and B-RAF activities paralleled their cell activities (P-AKT and P-ERK, respectively) and will not be discussed for simplification.
12. c-KIT FRET assay: Using a Fluorescence Resonance Energy Transfer (FRET) format, the c-KIT biochemical assay measured the ability of the receptor tyrosine kinase, c-KIT, to phosphorylate the generic substrate poly-(Glu,-Tyr)-biotin. For the generation of IC_{50} curves, the reaction was performed in 96-well opaque plates (Costar 3915) under the following conditions: a 10-mM stock solution of BAY compound (in 100% DMSO) was diluted 10-fold in 100% dimethylsulfoxide (DMSO). Compounds were then serially diluted, 1:5 for an eight-point dose curve, in 100% DMSO.A volume of 1 μL of the diluted compound was added to the reaction buffer. The reaction buffer was comprised of 50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.015% Brij35, 0.1 mg/ml BSA, and 0.1% β-mercaptoethanol. The following reagents were then combined and added to the reaction mixture: 10 nM poly-(Glu-Tyr)-biotin, 2 nM Eu-labeled phospho-tyrosine antibody (Perkin-Elmer PY20), and 10 nM streptavidin labeled APC (Perkin-Elmer). Next, ATP, at a final concentration of 5 μM, was added to the reaction mixture. Finally, the reaction was initiated with the addition of a recombinant, N-terminal GST fusion of human c-KIT (amino acids T_{544}–V_{976}, ProQinase) at a final concentration of 1.0 nM. The final reaction volume in each well was 100 μL and the final compound concentration ranges from 10 μM to 128 pM in 1% DMSO. The reaction mixture was allowed to incubate for 1 h after which time the reaction was terminated with the addition of staurosporine at a final concentration of 500 nM. The plates were read at 615 nM and 665 nM on a Perkin-Elmer Victor V Multilabel counter. Signal was calculated as a ratio: (665/615 nM) * 10,000. The data were expressed as percent inhibition: % inhibition = 100−((signal with inhibitor − background)/(signal without inhibitor − background)) * 100.
B-RAF biochemical assay: The B-RAF biochemical assay measures the ability of B-RAF kinase to phosphorylate MEK1. IC_{50} determinations were generated using the following reaction conditions: 50 mM Hepes, pH 7.5, 70 mM NaCl, 1 mM DTT, 8 mM $MgCl_2$, 80 ng/well B-RAF, 1 μg/well MEK1 substrate, and 5 μM ATP (0.2 μCi/well ^{33}P-ATP). Compounds were tested at 8 concentrations ranging from 10 μM to 4.5 nM with a final DMSO concentration in the assay of 1%. The reaction components were added to a COSTAR polypropylene U-Bottom 96-well plate and the reaction mixture was initiated with a stock ATP and $MgCl_2$ solution containing ^{33}P-ATP. The reaction mixture was then incubated for 1 h at 32 °C at which time adding phosphoric acid to a final concentration of 1% stopped the reaction. The reaction was then transferred to a filtermat via a Tomtec Harvester System. Filters were washed with 1% Phosphoric acid, rinsed with water, dried, and then sealed in bags with scintillation fluid. They were read on a Beta Plate Reader and data were analyzed with *Analyze 5* using a 4-parameter fit.
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