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3-Benzimidazol-2-yl-1*H*-indazoles as potent c-ABL inhibitors

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Abstract—The 3-benzimidazol-2-yl-1*H*-indazole scaffold was developed as an alternate scaffold for our receptor tyrosine kinase (RTK) inhibitor program. In exploring the SAR of this series, it was discovered that a subset of these compounds potently inhibit the enzyme c-ABL. The SAR of these compounds is described.

The tyrosine protein kinase, c-ABL, is ubiquitously expressed in mammalian cells and is involved in cell cycle regulation.[1] A chromosomal translocation between the bcr and abl genes results in a fusion protein with constitutive ABL kinase activity, leading to expansion of cells of the hematopoietic lineage and the disease, chronic myelogenous leukemia (CML).[2,3] The clinical development of imatinib mesylate (STI571, Gleevec™, **1**) has resulted in unprecedented response rates for the treatment of chronic phase CML patients.[4] Patients in the blast crisis stage of CML, however, have a less durable response.[4] The primary mechanism of acquired Gleevec™ resistance occurs through kinase domain mutations in BCR-ABL.[5] Other small molecule inhibitors are currently under development to treat Gleevec™-resistant CML.[5]

During the course of our RTK program, we desired an alternate scaffold to the 4-amino-3-benzimidazol-2-yl-hydroquinolin-2-one series (Fig. 1, **2**).[6–8] The 3-benzimidazol-2-yl-1*H*-indazole (**3**, hereafter referred to as indazole benzimidazole) series was designed, and the SAR explored.[9] During the course of that work, a subset of compounds was identified as potent c-ABL inhibitors. This SAR was developed and is presented here.

In evaluating the indazole benzimidazole scaffold, a number of substituents were assessed on the D ring of the benzimidazole, and, when located at C5', most substituents were found to be tolerated. Typically, basic amines improved physiochemical properties, and thus were evaluated more closely. Compounds containing the piperidinylpiperidine amine inhibited other RTKs potently and exhibited good inhibition of cellular proliferation.[9] This moiety, then, was used in expanding the SAR around the subset of compounds shown to exhibit potent c-ABL activity. Compound **4** (Table 1), with no functionality on ring A, moderately inhibits c-ABL phosphorylation at 0.60 μM similar to the potency exhibited by Gleevec™ (**1**, 0.43 μM). Analysis of the SAR of the indazole benzimidazoles indicated that c-ABL affinity was dependent on being substituted at C5 or C6.[10] Incorporating a methoxy at C-5 (**5**) improves the in vitro potency against c-ABL somewhat, but a nearly 10-fold improvement is seen when the much larger benzyloxy (**7**) moiety is incorporated at C5. This improvement in affinity increases to 19-fold when C5 is substituted with phenoxy (**6**). Furthermore, a 200-fold improvement in enzymatic inhibition is seen when a methoxy is incorporated at C6, ortho to the C-5 benzyloxy, (**8**). Attempts to incorporate two large groups such as in compound **10** (5,6-dibenzyloxy) or move the benzyloxy substituent to C6 as in compound **9** result in significant loss of affinity compared to **8**.

Because of the observation that a large substituent at C5 of the indazole ring significantly improved potency against c-ABL, effects of substitution on the phenoxy were evaluated. Incorporation of a methoxy on the ortho- positon of the phenoxy ring (**11**) yielded similar affinity as the unsubstituted phenoxy (**6**), while

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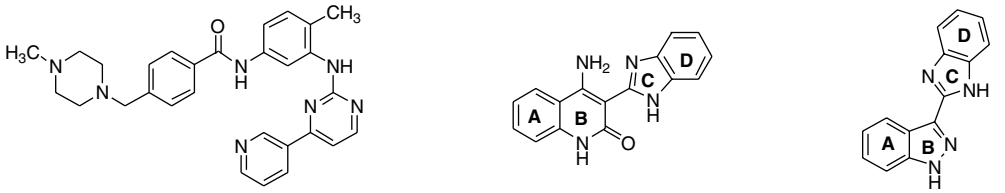
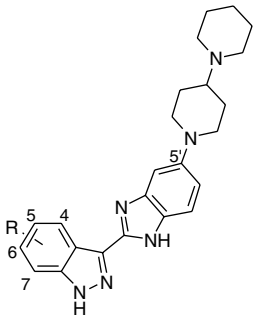
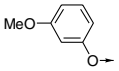
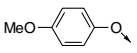
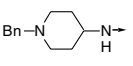
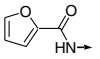
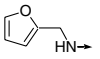
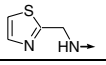


1 2 3

Figure 1. Imatinib mesylate (Gleevec™, **1**), 4-amino-3-benzimidazol-2-ylhydroquinolin-2-one (**2**), and 3-benzimidazol-2-yl-1*H*-indazole (**3**).

Table 1. Structure–activity relationship of the indazole benzimidazole series against c-ABL



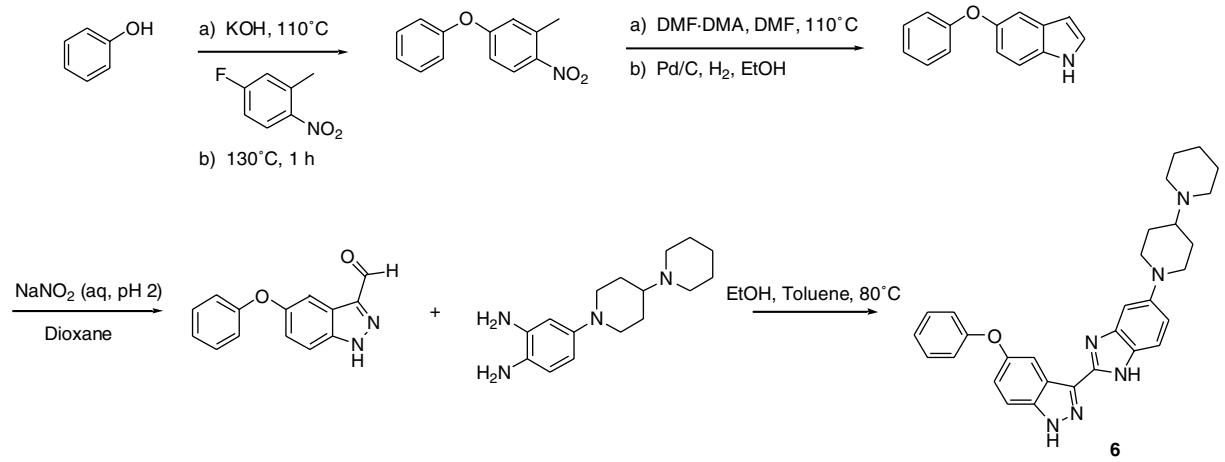
Compound	R	c-ABL IC_{50} (μM)
1 (Gleevec™)	—	0.43
4	H	0.60
5	5-OMe	0.23
6	5-OPh	0.03
7	5-OBn	0.06
8	5-OBn, 6-OMe	0.003
9	6-OBn	0.20
10	5-OBn, 6-OBn	0.90
11	5- 	0.02
12	5- 	0.06
13	5- 	0.22
14	5-NHBn	0.13
15	5- 	0.007
16	5-NH(CO)NH*t*-Bu	0.03
17	5- 	0.15
18	5- 	0.06
19	5- 	0.01

substitution at the meta- (**12**) and para-positions (**13**) led to a decrease in activity. A similar trend was seen with a fluoro substituent.[10]

Analogs with amino-linked substituents at C-5 were synthesized and evaluated. The benzylamino substituted analog (**14**) was less potent than the oxy analog (**7**), but the more structurally constrained 1-benzyl-4-piperidylamine (**15**) exhibited potent enzymatic inhibitory activity. The *tert*-butyl urea moiety (**16**) also conferred potent enzymatic affinity to the molecule. The amide (**17**) showed less potent c-ABL inhibition, but removal of the carbonyl to give 2-furylmethylamine (**18**) improved affinity. And, finally, replacement of the furan (**18**) with a thiazole (**19**) gave an additional 5-fold potency improvement.

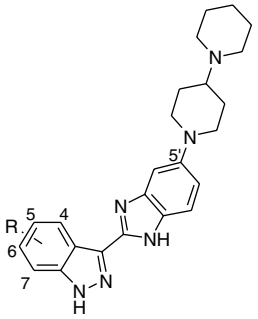
A subset of the analogs screened in the enzymatic assay were further evaluated in the CML cell line K562 which expresses the Bcr-Abl protein (Table 2).[11] The 5-benzyl analog (**7**) inhibited K562 cellular proliferation with an EC_{50} of 0.97 μM which was 11-fold less potent than Gleevec™ (**1**, 0.09 μM). The amino-linked benzyl analog (**14**) exhibited poorer cell growth inhibition than **7**, while the 1-benzyl-4-piperidylamine analog (**15**) had 14-fold improvement in cellular inhibitory activity compared to **7** and was on par with that of Gleevec™'s (**1**). The *tert*-butyl urea compound (**16**) had slightly improved cell potency compared to **7** but significantly worse cellular potency compared to **15**. Comparing **17**, **18**, and **19**, the anti-proliferative effect followed the same trend as the enzymatic data in that the 2-furylmethylamine (**18**) was more potent against the K562 cells than **17**, while the 2-thiazolylmethylamine (**19**) is the most potent analog tested on this cell line. The potency of **18** on cell is greater than the c-ABL in vitro activity, while the cellular potency of **19** is equipotent to the enzymatic affinity indicating that the inhibition of K562 proliferation is most likely influenced by potency against other kinases.[12]

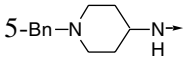
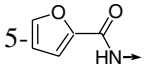
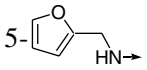
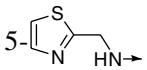
Synthesis of the indazole benzimidazole core from an indazole aldehyde and a phenylenediamine has been previously described.[9] For the aryloxy analogs (Scheme 1, **6**, **11**–**13**), the indoles were synthesized by S_NAr of the anion of phenol or substituted phenol on 4-fluoro-2-methyl-1-nitrobenzene followed by condensation with DMF · DMA, reduction, and in situ cyclization to the indole.[13,14] The anion of phenol (and substituted phenols) could be made by combining the molten phenol with KOH at 110 °C.[13] The indazole aldehydes were synthesized as previously described.[9,15,16] For the nitrogen-linked analogs (**14**–**19**), the nitro was carried



Scheme 1. Synthetic scheme for the 5-phenoxy-substituted indazole benzimidazole, **6**.

Table 2. Cellular proliferation data for selected compounds



Compound	R	c-ABL IC_{50} (μM)	K562 EC_{50} (μM)
1 (Gleevec™)	—	0.43	0.09
7	5-OBn	0.06	0.97
14	5-NHBn	0.13	1.8
15		0.007	0.07
16	5-NH(CO)NH*t*-Bu	0.03	0.36
17		0.15	0.56
18		0.06	0.03
19		0.01	0.01

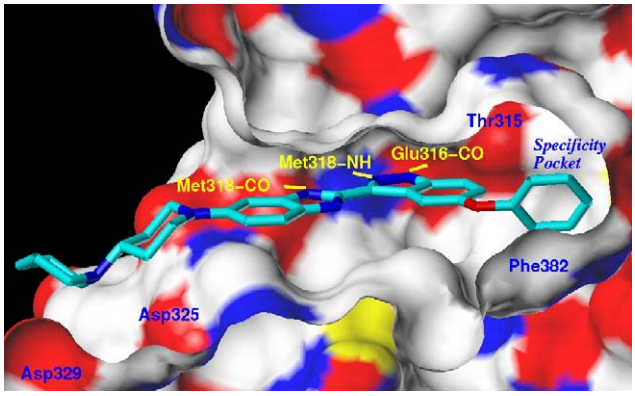


Figure 2. Compound **6** docked at the ATP binding site of c-ABL. The protein structure is taken from Brookhaven Protein Data Bank, entry 1M52, where the kinase adopts an inactive conformation with the P-loop residues wrapping around the ATP site (not shown in the picture) and the Phe382 flanking out at the DFG loop. Surface of the binding site is colored by atom-type (Oxygen, red; Nitrogen, blue; and Carbon, white), and hydrogen bonds to hinge residues are also labeled.

through the reaction sequence from the commercially available 5-nitroindole. Subsequent catalytic reduction followed by reaction with an electrophile (**16**–**17**) or by reductive amination (**14**–**15** and **18**–**19**) gave the desired products.

Figure 2 shows a docking model of compound **6** at the ATP binding site of c-ABL. In this proposed binding mode, the indazole benzimidazole core of compound **6** forms three H-bonds with residue Glu316 and Met318 at the hinge of the kinase. The basic piperidinopiperidine group of the ligand stretches into the solvent and is in close contact with two acidic residues Asp325 and Glu329. The phenoxy group at C5 occupies the kinase specificity pocket consisting mainly of the side chains of Val256, Met290, Phe382, Ile313, Glu286, and Lys271. Although, this docking model could explain the general SAR that C5 substitution leads to more active compounds, it is not representative of all the C5 substituted compounds. For example, one of the more active compounds, **15**, would probably bind to a different conformation of the enzyme (model shown in Supplemental Material). It is also worth noting that this series of compounds do not seem to form the H-bond with the side chain of Thr315 as Gleevec™ does, therefore they are expected to overcome one of the major Gleevec™-resistance mutations, T315I.[17]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.043.

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