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Imidazo[4,5-*c*]quinolines as inhibitors of the PI3K/PKB-pathway

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Abstract—Imidazo[4,5-*c*]quinoline derivatives have been discovered and developed as potent and effective modulators of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) pathway to lead to clinical development candidates. The SAR data of representative examples of this compound class and their biological profiling in cellular and in vivo settings are presented and discussed.

The PI3K/PKB-pathway is one of the major signaling pathways activated in cancer via deletion, activating mutation or amplification of one or several of its components. The node of the pathway is PKB (also called Akt), a serine/threonine protein kinase responsible for the phosphorylation of downstream effectors involved in important cellular processes such as metabolism, growth, proliferation, survival, and also angiogenesis. Activation of PKB requires three sequential events: recruitment at the membrane by phosphatidylinositol-3,4,5-trisphosphate (PIP3), the product of the lipid kinase PI3K which is negatively regulated by the tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN); phosphorylation of Thr-308 (PKBα numbering) by the 3-phosphoinositide-dependent protein kinase-1 (PDK1); and phosphorylation of Ser-473 (PKBα numbering) by mTORC2, which is a complex formed by mTor and rictor.[1] Given the role of the PI3K/PKB-pathway in the biology of human cancers, some of the components of this signaling cascade have become the focus of intensive drug discovery activities, mostly still at preclinical stage.[2]

The imidazo[4,5-*c*]quinoline scaffold (Fig. 1a) can be considered a privileged ATP-site-directed kinase lead structure for drug discovery activities. This chemotype can adopt different binding modes in the ATP binding cleft, to mimic the H-bond interactions of the adenine moiety of ATP (Fig. 1b). The structural elements of the compound and its binding mode can be exploited to reach regions of the active site unique to the targeted

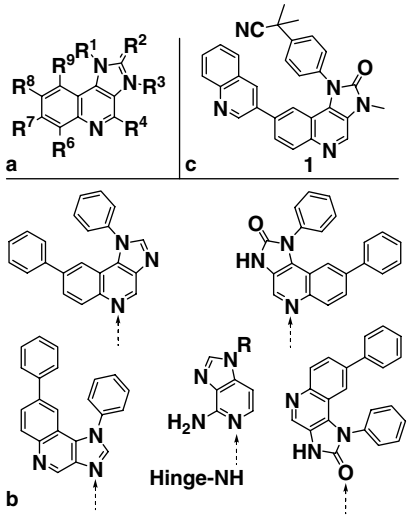

Figure 1. (a) Generic structure of the imidazo[4,5-*c*]quinoline scaffold; (b) different potential binding modes mimicking the canonical H-bond interactions of adenine with the hinge region; (c) NVP-BEZ235 (**1**).

kinase, in order to achieve the desired activity and selectivity profile. This scaffold has been used and optimized for the identification of new modulators of the PI3K/PKB-pathway, leading to the discovery and development of NVP-BEZ235 (**1**) (Fig. 1c). This compound is a dual PI3K/mTor inhibitor, which is currently undergoing Phase I clinical trials in cancer patients.

We report herein our initial efforts to identify modulators of the PI3K/PKB-pathway, and the in vitro and in vivo biological characterization of representative examples of PDK1 and PI3K kinase inhibitors based on the imidazo[4,5-*c*]quinoline scaffold.

Keywords: PDK1; PI3K; Oncology; PI3K/PKB-pathway.
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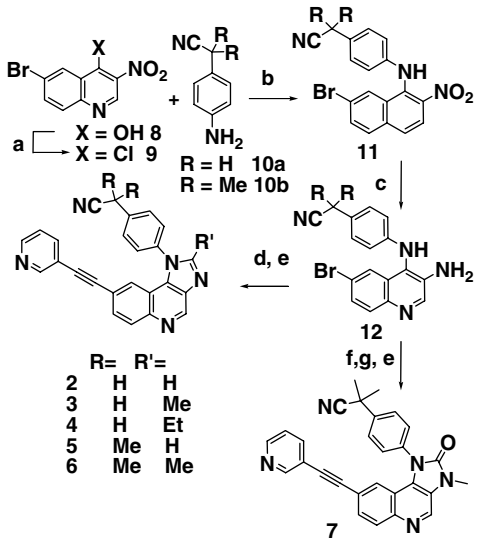

Scheme 1. Reagents and conditions: (a) $POCl_3$, 6 h at 125 °C; (b) AcOH, 1 h at rt; (c) H_2, cat. Ni/Ra, MeOH/THF, 12 h at rt; (d) methyl ortho ester, 1–2 h at 110–130 °C; (e) 3-acetyleneylpyridine, cat. $Pd(PhCN)_2Cl_2$, $^tBu_3P$, CuI, dioxane/NMP, 0.5–3 d at rt; (f) diphosgene, NEt_3, CH_2Cl_2, 20 min at 0 °C; (g) MeI, $NaOH_{(aq)}$, cat. TBABr, CH_2Cl_2, 18 h at rt.

Table 1. Inhibition of PDK1 kinase activity in biochemical assays and phosphorylation of T308-PKB in cellular settings, and anti-proliferative activity

Compounds	PDK1 IC_{50}[a] (nM)	p-T308-PKB IC_{50}[b] (nM)	Proliferation EC_{50}[c] (nM)
2	34	94	416
3	110	77	275
4	327	138	400
5	150	139	446
6	245	45	143
7	>25,000	33	13

[a] Enzymatic activity with ATP concentration at 10 μM.
[b] In vitro cell capture ELISA in U87MG.
[c] In vitro antiproliferation on U87MG.

Starting from a previously identified imidazo[4,5-*c*]quinoline based hit, compound **2** (Scheme 1) was shown to be a potent inhibitor of PDK1 (IC_{50} = 34 nM; Table 1). PDK1 is a serine/threonine kinase belonging to the AGC kinase family. In addition to its catalytic domain, this protein has a C-terminal pleckstrin homology (PH) domain required for the binding of PIP3. PDK1 is uniquely responsible for a PI3K-dependent activating phosphorylation of PKB, but other kinases of the AGC family can also be activated by PDK1 in a PI3K-independent manner.[3] Compound **2** has a good selectivity profile against other protein kinases, including other members of AGC kinase family (e.g., PKA and PKB, 24% and 0% inhibition at 10 μM, respectively), but **2** is active against class I PI3K (IC_{50} = 64, 432, 98, and 67 nM for p110α, p110β, p110δ, and p110γ, respectively). This family of lipid kinases is composed of two subgroups, IA and IB. The class IA PI3K subgroup consists of three catalytic subunits, p110α, p110β, and p110δ, all of which interact with regulatory subunits allowing for their recruitment by an activated receptor tyrosine kinase. The class IB PI3K consists of p110γ which is principally recruited by GPCR via its regulatory subunit.[4] The most relevant paralog for cancer therapy is probably the p110α protein, for which activating mutations have been identified in tumor clinical samples.[5] The physiological role of the class I PI3K isoforms has been discussed elsewhere.[6]

Compounds **2**–**7** were efficiently obtained starting from 6-bromo-4-hydroxy-3-nitroquinoline (**8**) (Scheme 1). The position 4 of quinoline **8** is activated via chlorination using phosphoroxychloride to yield **9**, followed by an acid promoted S_NAr reaction with aniline **10a** or **10b**. The nitro-intermediate **11** is subjected to Raney nickel catalyzed hydrogenation to afford intermediate **12**. Closure of the 5-membered ring of the imidazoquinoline system is achieved by heating **12** in neat methyl orthoformate, acetate or propionate with reaction time and temperature requirements increasing with the homologation of the ortho ester. The resulting bromo-intermediates are subjected to a Sonogashira coupling reaction to afford compounds **2**–**6**.

A slightly modified protocol was used for the synthesis of compound **7**.[7] Closure of the imidazolinone ring is achieved with diphosgene in the presence of triethylamine, followed by tetrabutylammonium bromide phase transfer catalyzed methylation at position 3. As for compounds **2**–**6**, a Sonogashira coupling reaction following a modified protocol using the bulky tri-*tert*-butylphosphine as ligand[8] provides the target compound. Aniline **10b** is obtained from 4-nitrophenylacetonitrile in two steps (in a similar manner as described for steps g and c, Scheme 1).

Modification of the lead compound **2** by introducing a methyl (compound **3**) or ethyl (compound **4**) group at position 3 decreased the PDK1 kinase inhibitory activity by up to one log unit (Table 1). Replacing the potential metabolic weak spot and hydrogen bond acceptor methylcyano moiety with a dimethyl methylcyano group (compound **5**) had a small detrimental effect on PDK1 activity as shown in Table 1. Combining the dimethyl methyl cyano substituent with a methyl group on the imidazole ring gave compound **6**. This derivative has a reduced PDK1 inhibitory activity, but remains equipotent against PI3K (IC_{50} = 56, 446, 35, and 117 nM for p110α, p110β, p110δ, and p110γ, respectively) as compared to compound **2**. A good correlation was observed between the inactivation of the pathway, as measured by inhibition of phosphorylation of Thr308 of PKB (p-T308-PKB), and the anti-proliferative activity of the inhibitor (Table 1). The functional activity of compound **6** was further assessed in cellular settings.

Tumor cell lines, in which the PI3K/PKB-pathway is known to be deregulated via PTEN deletion (U87MG glioblastoma and PC3M prostate tumor cells), were exposed to increasing concentrations of compound **6** and cell extracts were monitored by Western blot (Fig. 2). A dose-dependent decrease of p-PKB was observed in both tumor cell lines, whereas the total PKB levels stayed constant, demonstrating that the effect on p-

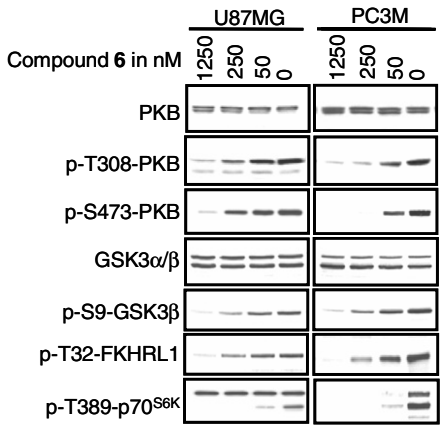

Figure 2. Western blot analysis of U87MG and PC3M cell extract treated with increasing concentrations of compound **6**.

PKB could not be due to protein degradation. Inhibition of activation of PKB was associated with a reduction in the levels of phosphorylated direct or indirect downstream effectors like Ser9-GSK3β, Thr32-FKHRL1/FOXO3a, and Thr389-p70^{S6K}, showing efficient PI3K/PKB-pathway blockade upon in vitro compound treatment. The in vivo efficacy of compound **6** was assessed using PC3M tumors grown subcutaneously in athymic mice. The rate of tumor growth was reduced and tumor volumes measured at day 15 corresponded to a non-statistically significant treatment/control (*T*/*C*) ratio of 50% at the highest dose tested, 75 mg/kg bid daily po (Fig. 3).

Further modification of compound **6** by changing the imidazo ring to *N*-methyl imidazolinone (compound **7**) resulted in a complete loss in PDK1 inhibitory activity. In spite of this, compound **7** retained its activity against PI3K (IC_{50} = 72, 2336, 201, and 382 nM for p110α, p110β, p110δ, and p110γ, respectively) and effectively

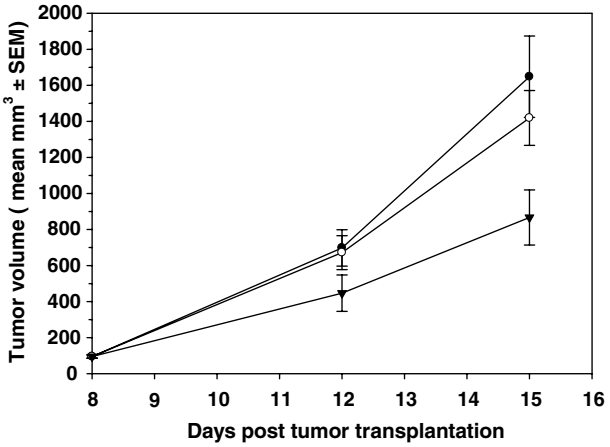

Figure 3. In vivo efficacy of compound **6**. PC3M tumor bearing animals were treated with compound formulated in NMP/PEG300, po at a dose of either 50 (open circles) or 75 (dark triangles) mg/kg, twice per day. The antitumor activity measured as *T*/*C* (mean difference of treated group divided by the mean difference of vehicle treated control group (dark circles), multiplied by 100) on day 15 was 85% and 50%, respectively.

blocked the activation of the pathway (IC_{50} = 33 nM, p-T308-PKB; Table 1). Analysis of the docking of compound **2** in the PDK1 active site shows a short distance (3.4 Å) between the carbon in position 2 of the imidazole ring and the backbone carbonyl of Leu88 of the P-loop (Fig. 4a). In agreement with the in vitro PDK1 kinase activity profile, a methyl (compounds **3** and **6**) or ethyl (compound **4**) substituent resulted in a slight steric demand, but introduction of a carbonyl group at position 2 (compound **7**) created an electrostatic mismatch. Such a strong repulsive interaction is not present in the PI3K active site because in this lipid kinase the structural motif corresponding to the P-loop of protein kinases has a different spatial arrangement with no residue matching the position of Leu88 in PDK1. As a consequence, the carbonyl group of compound **7** is facing the protein–water interface solvent with Trp812 and Met804—the closest residues of the loop—being located above this group (Fig. 4b).

The increase in the cellular potency of compound **7** as compared to **6** also translates into improved in vivo efficacy. At an equivalent dosing schedule of 50 mg/kg bid daily, compound **7** achieved a statistically significant *T*/*C* value of 31% versus the 80% value obtained with compound **6**. Almost tumor stasis (*T*/*C* of 9%) is achieved at 60 mg/kg with the same schedule (Fig. 5). Ex vivo anal-

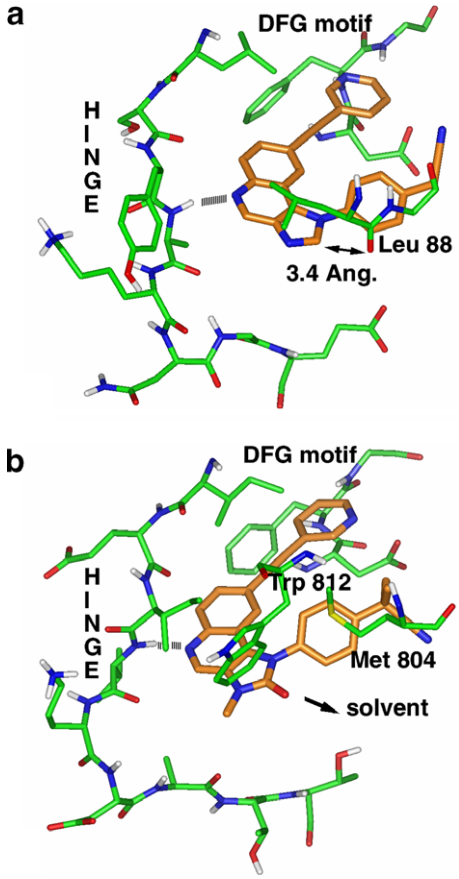

Figure 4. (a) Compound **2** docked in the X-ray co-crystal structure of PDK1 with ATP (PDB code 1H1W) as compared to (b) the docking of compound **7** in the X-ray co-crystal structure of PI3Kγ with staurosporine (PDB code 1E8Z).

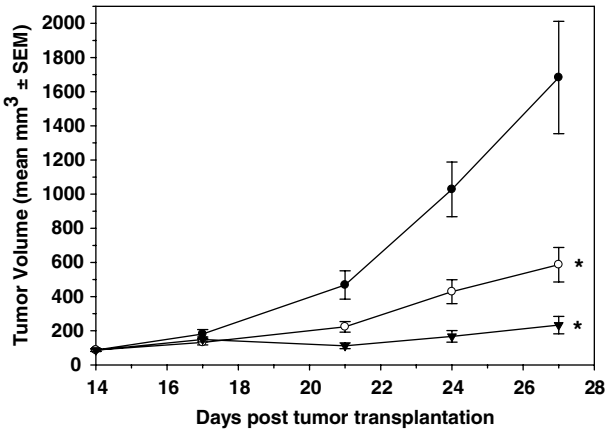

Figure 5. Efficacy of compound **7**. PC3M tumor bearing animals were treated with compound formulated in NMP/PEG300, po at a dose of either 50 (open circles) or 60 (dark triangles) mg/kg, twice per day. The antitumor activity measured as *T*/*C* (mean difference of treated group divided by the mean difference of vehicle treated control group (dark circles), multiplied by 100) on day 27 was 31 and 9%, respectively. $^{*}p < 0.05\%$ versus control, one way ANOVA followed by post hoc Dunnet's test.

yses of tumor tissue after a single dose administration (50 mg/kg po) showed a time-dependent correlation between compound concentration and PKB phosphorylation inhibition for compounds **6** and **7**, but the latter displaying a lower maximal concentration than the former (data not shown).

In conclusion, we have demonstrated the ability of a series of imidazo[4,5-*c*]quinoline derivatives—compounds **2**–**7**—to inhibit the kinase activity of components of the PI3K/PKB-pathway. The effect of their inhibitory activity on PDK1 and/or PI3K can be tracked to the cellular inactivation of PKB and blockade of the pathway. This biological effect correlates with a significant antiproliferative activity against tumor cell lines in which the PI3K/PKB-pathway is known to be deregulated via PTEN deletion. The results obtained with the dual PDK1/PI3K inhibitors show that concurrent inhibition of these enzymes is an effective strategy to block the phosphorylation and activation of PKB. However, inhibition of PI3K activity, as illustrated with compound **7**, is also sufficient to control PKB activation in cellular and in vivo settings. The lack of a selective PDK1 inhibitor makes the evaluation of the PDK1 contribution to the efficacy of the dual inhibitors difficult, but the simultaneous blockade of PDK1 and PI3K kinases by the same compound may provide a therapeutic advantage in terms of avoiding or delaying the appearance of drug resistance. It is unclear at this moment if a specific activity profile against one or several of the components of the pathway will maximize antitumor activity and tolerability.

Compounds **6** and **7** have demonstrated their ability to reduce tumor growth in an in vivo mouse model. Furthermore, both compounds provide good starting points to explore the possibility of developing dual PI3K/PDK1 or selective PI3K inhibitors. Progress in these series will be reported in due course.

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7. Analytical data for compound **7**: ^{1}H NMR ($(CD_3)_2SO$, 600 MHz) δ 9.04 (s, 1 H, H-4), 8.61 (m, 1 H, H-2 pyridine), 8.57 (dm, 1 H, $J = 4.9$ Hz, H-6 pyridine), 8.05 (d, 1 H, $J = 8.7$ Hz, H-6), 7.87 (AA′, 2 H, H-3,5 phenyl), 7.84 (dt, 1 H, $J = 7.8$ Hz, $J = 1.8$ Hz, H-4 pyridine), 7.72 (BB′, 2 H, H-2,6-phenyl), 7.65 (dd, 1 H, $J = 8.7$ Hz, $J = 1.7$ Hz, H-7), 7.44 (dd, 1 H, $J = 7.9$ Hz, $J = 4.9$ Hz, H-5 pyridine), 7.04 (m, 1 H, H-9), 3.60 (s, 3 H, CH_3), 1.79 (s, 6 H, $(CH_3)_2$). ^{13}C NMR δ 152.90, 151.42, 149.28, 143.73, 138.42, 134.57, 134.43, 130.86, 129.44, 128.40, 128.26, 127.03, 124.40, 124.11, 123.63, 123.54, 118.85, 118.84, 114.36, 91.87, 86.95, 36.76, 28.15, 27.75. HR-MS: Calcd for $C_{28}H_{22}N_5O^+$: 444.18189. Found: 444.18186.
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