Discovery of Tetrahydropyridopyrimidines as Irreversible Covalent Inhibitors of KRAS-G12C with In Vivo Activity

Jay Bradford Fell, John P Fischer, Brian R Baer, Joshua Ballard, James F Blake, Karyn Bouhana, Barbara J Brandhuber, David M Briere, Laurence E. Burgess, Michael R Burkard, Harrah Chiang, Mark J Chicarelli, Kevin Davidson, John J Gaudino, Jill Hallin, Lauren Hanson, Kenneth Hee, Erik J Hicken, Ronald J Hinklin, Matthew A. Marx, Macedonio J Mejia, Peter Olson, Pavel Savechenkov, Niranjan Sudhakar, Tony P Tang, Guy P. Vigers, Henry Zecca, and James G. Christensen

ACS Med. Chem. Lett., Just Accepted Manuscript • DOI: 10.1021/acsmedchemlett.8b00382 • Publication Date (Web): 07 Nov 2018

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.
Discovery of Tetrahydropyridopyrimidines as Irreversible Covalent Inhibitors of KRAS-G12C with In Vivo Activity

Jay B. Fell*1, John P. Fischer1, Brian R. Baer1, Joshua Ballard1, James F. Blake1, Karyn Bouthana1, Barbara J. Brandhuber1, David M. Briere2, Laurence E. Burgess1, Michael R. Burkard1, Harrah Chiang2, Mark J. Chicarelli1, Kevin Davidson1, John J. Gaudino1, Jill Hallin2, Lauren Hanson1, Kenneth Hee1, Erik J. Hicken1, Ronald J. Hinklin1, Matthew A. Marx2, Macedonio J. Mejia1, Peter Olson2, Pavel Savechenkov1, Niranjan Sudhakar2, Tony P. Tang3, Guy P. Vigers1, Henry Zecca1, James G. Christensen2

1Array BioPharma Inc., 3200 Walnut St, Boulder, Colorado, 80301.
2Mirati Therapeutics Inc., 9393 Towne Centre Drive, Suite 200, San Diego California, 92121.

KEYWORDS Cancer, KRAS, G12C, covalent

ABSTRACT: KRAS is the most frequently mutated driver oncogene in human cancer and KRAS mutations are commonly associated with poor prognosis and resistance to standard treatment. The ability to effectively target and block the function of mutated KRAS has remained elusive despite decades of research. Recent findings have demonstrated that directly targeting KRAS-G12C with electrophilic small molecules that covalently modify the mutated codon 12 cysteine is feasible. We have discovered a series of tetrahydropyridopyrimidines as irreversible covalent inhibitors of KRAS-G12C with in vivo activity. The PK/PD and efficacy of compound 13 will be highlighted.

KRAS is the single most frequently mutated oncogene and the first of more than 700 genes to be causally implicated in human cancer (COSMIC).1 Mutations in KRAS are prevalent amongst the top three most deadly cancer types in the United States: pancreatic (95%), colorectal (45%), and lung (35%).2 The frequent mutation of KRAS across a spectrum of aggressive cancers has stimulated an intensive drug discovery effort to develop therapeutic strategies that block KRAS function for cancer treatment. Despite nearly four decades of research, a clinically viable KRAS cancer therapy has remained elusive. However, recent findings have stimulated a new wave of activities to develop KRAS targeted therapies.

Capping off an era marred by drug development failures and punctuated by waning interest and presumed intractability toward direct targeting of KRAS, new technologies and strategies are aiding in the target’s resurgence.3,4 Central to this renewal, is a single mutation: KRAS-G12C, a well-validated driver mutation and the most frequent individual KRAS mutation in lung cancer.5 Associated with poor prognosis and resistance to treatment, KRAS-G12C represents both an extraordinary unmet clinical need and opportunity. This mutation has a causal role in 14% of lung adenocarcinomas (~14,000 new US cases annually), 5% of colorectal adenocarcinomas (~ 5,000 new US cases annually) and smaller fractions of other cancers. Collectively, KRAS-G12C mutations comprise a patient population with a worldwide annual incidence of greater than 100,000 individuals. The scientific basis for targeting KRAS-G12C was originally described in a breakthrough article by Shokat and colleagues.6 In their paper the authors reported the identification of compounds that bound to a previously unappreciated pocket near the KRAS switch II effector region. The compounds form an irreversibly covalent bond to the mutant cysteine 12, locking the protein in its inactive GDP-bound state.7 Targeting the switch II binding site was a clear advance in the field, this communication describes the discovery of covalent small molecule KRAS-G12C inhibitors with in vivo activity.

The Array BioPharma covalent fragment collection was screened against KRAS-G12C in a protein modification assay similar to that described by Ostrem.8 Subsequent hit elaboration was performed in an iterative library format. Primary observations from this exercise were incorporated into a structure based drug design effort which led to the discovery of compound 4. Our methods were validated by the publication of papers containing other KRAS-G12C inhibitors.8,9
Tyr96, Ile100, and Val103. The side chain carboxylate of Asp69 is positioned ca. 3.3 Å from the naphthyl ring of 4. While Asp69 forms a salt bridge with Arg102, it may be possible to form an additional H-bond to this carboxylate.

Figure 1 illustrates the X-ray crystal structure of 4 in complex with KRAS-G12C at 1.8 Å resolution, hydrogens added for clarity (PDB code XXXX).

Table 1. Comparison of Naphthyl Replacement SAR

<table>
<thead>
<tr>
<th>Compound</th>
<th>POC Mod.</th>
<th>H358 IC&lt;sub&gt;50&lt;/sub&gt; µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>13%</td>
<td>&gt;16</td>
</tr>
<tr>
<td>5</td>
<td>22%</td>
<td>&gt;16</td>
</tr>
<tr>
<td>6</td>
<td>2%</td>
<td>&gt;16</td>
</tr>
<tr>
<td>7</td>
<td>2%</td>
<td>&gt;16</td>
</tr>
<tr>
<td>8</td>
<td>99%</td>
<td>7.8</td>
</tr>
<tr>
<td>9</td>
<td>0%</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

In vitro activity assays. Percent of control (POC) protein modification assay measured at the 3 hr timepoint with 5 µM compound. H358 cell assay IC<sub>50</sub> showing concentration at which 50% suppression of ERK phosphorylation is observed. Please see supporting information for standard deviation and the number of tests.

Table 1 describes a series of analogs that were synthesized in an attempt to make an H-bond to Asp69, the POC data at 3 hr/5 µM as well as the H358 cell IC<sub>50</sub> data is presented. Compound 4 with no H-bond donor is included for reference. Indazole 5 was equipotent to naphthyl in the POC assay and had no activity in the cellular assay. As noted above, the naphthyl region of the binding pocket is highly lipophilic...
suggesting that loss of contacts in this region would lead to
significant potency decreases (i.e., naphthyl to phenyl
substitution). This result was borne out experimentally; the
two phenol compounds 6 and 7 show 2% protein
modification in the 3 hr/5 µM conditions. Naphthol
compound 8 was the most potent compound in this series
with 99% protein modification and measurable cell activity
with an IC_{50} = 7.6 µM. Finally, compound 9 showed 0%
modification under these assay conditions; presumably due
to poor positioning of the hydroxyl group.

Table 2. Exploration of the Basic C-2 Side Chain

<table>
<thead>
<tr>
<th>R_1</th>
<th>POC Mod. (15 min/3 µM)</th>
<th>H358 IC_{50} µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>8%</td>
<td>7.6</td>
</tr>
<tr>
<td>10</td>
<td>52%</td>
<td>1.9</td>
</tr>
<tr>
<td>11</td>
<td>22%</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>21%</td>
<td>0.54</td>
</tr>
<tr>
<td>13</td>
<td>84%</td>
<td>0.070</td>
</tr>
</tbody>
</table>

In vitro activity assays. Percent of control (POC) protein modification assay measured at the 15 min timepoint with 3 µM compound. H358 cell assay IC_{50}
showing concentration at which 50% suppression of ERK phosphorylation is
observed. Please see supporting information for standard deviation and the
number of tests.

We hypothesized that substitution at the C-2 position of the
pyrimidine ring would afford access to the carboxylate of
Glu62. Shown in Table 2, potential ionic interactions with
Glu62 were tested with compounds 10 and 11 by varying the
chain length to place a basic amine near the carboxylate.
Both of these compounds were more active in the protein
modification assay, at the 15 min/3 µM conditions, and cell
assay compared to compound 8 (included in Table 2 for
comparison). The rigidified α-methyl analog 12 showed a
further increase in cell potency.

A high-resolution crystal structure of compound 12 bound to
KRAS-G12C is shown in Figure 2. The binding mode for 12 is
similar to that of compound 4. Interestingly, even though the
KRAS protein was reacted with racemic compound 12 in
solution, only the (R)-enantiomer was seen in the crystal
structure. Docking studies suggested that the (S)-enantiomer
would display more strain energy in the bound conformation.

In addition to the previously mentioned interactions, there is
a 2.65 Å H-bond from the naphthyl OH to the carboxylate of
Asp69. The C-2 amino substituent forms a salt-bridge with
the carboxylate of Glu62, with an N-O distance of 2.80 Å, and
a cation-π interaction with His95 (His95 ring centroid to
amine nitrogen distance is 5.21 Å). Based on this X-ray
structure and the observed amine interactions with Glu62
and His95, we designed compound 13 with the aim of
eliminating one rotatable bond, and the introduction of more
hydrophobic contacts with His95. Docking studies of
compound 13 suggested that the pyrrolidine ring amine
would make the same salt-bridge interaction with Glu62 and
cation-π interaction with His95. The N-methyl pyrrolidine C-2
amine substituent of compound 13 displayed a dramatic
boost in potency, protein modification POC = 84% and cell
IC_{50} = 0.070 µM. We attributed this potency increase to the
aforementioned interactions and the removal of one
rotatable bond.

The importance of the RAS-MAPK pathway was mentioned
earlier; PI3K is another effector pathway of RAS, regulating
cell growth, cell cycle entry, cell survival and metabolism.12
Both of these pathways play key roles in the physiology of
healthy cells such that it is of critical importance for a KRAS-
G12C inhibitor to spare wild type (WT) KRAS. In order to
determine selectivity for the KRAS-G12C mutation, the
activity of compound 13 against three non-G12C cell lines was
determined. The cell lines studied included the KRAS-WT
RKO and SNU-C5 lines as well as the KRAS-G12D AGS line.
In all three lines compound 13 was found to be inactive with
IC_{50} exceeding 16 µM, the highest concentration tested.
To determine if compound 13 was a suitable candidate for in vivo anti-tumor efficacy studies, the ADME and PK properties were evaluated. Compound 13 displayed low permeability with P-gp efflux in the MDR1 transfected LLC-PK1 cell permeability assay, and mouse plasma protein binding (PPB) was 95% (Table 3). The predicted hepatic clearance (CL\text{h}) in mouse was calculated to be 53 mL/min/kg and 76 mL/min/kg from compound 13 half-life data in mouse liver microsomes and mouse hepatocytes incubations, respectively. The lower stability in hepatocytes indicated the involvement of Phase II metabolism. Following a 3 mg/kg IV dose of compound 13 to CD-1 mice, the clearance was 46 mL/min/kg (Table 4). Oral administration of 100 mg/kg compound 13 to mice resulted in a C\text{max} of 0.59 µg/mL (Table 4), which was below the calculated free fraction adjusted cellular IC\text{50} of 1.4 µM (0.74 µg/mL). Although the PK parameters predicted to drive antitumor efficacy were not known, PO dosing did not appear to warrant further evaluation in an efficacy study. However, the moderate clearance of compound 13 supported intraperitoneal (IP) dosing as an opportunity to demonstrate anti-tumor activity. IP dosing resulted in appreciably higher concentrations of compound 13 in mouse plasma compared to oral dosing. Following the 15 or 50 mg/kg doses, drug plasma concentrations were above the free fraction adjusted IC\text{50} for 1-3 hr. However, the 100 mg/kg dose provided >6 hr coverage of the free fraction adjusted cellular IC\text{50} (Table 5). Based on these results, 30 mg/kg and 100 mg/kg IP doses were selected for tumor growth inhibition experiments.

Table 3. ADME Properties of Compound 13

<table>
<thead>
<tr>
<th>ADME Assay</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 transfected LLC-PK1 Permeability</td>
<td>P\text{app} (A to B) = 1.4 x10^{-6} cm/sec</td>
</tr>
<tr>
<td></td>
<td>P\text{app} (B to A) = 37 x10^{-6} cm/sec</td>
</tr>
<tr>
<td></td>
<td>BA/AB Ratio = 27</td>
</tr>
<tr>
<td>Mouse PPB</td>
<td>95%</td>
</tr>
<tr>
<td>Mouse Predicted CL\text{h} (microsomes)</td>
<td>53 mL/min/kg</td>
</tr>
<tr>
<td>Mouse Predicted CL\text{h} (hepatocytes)</td>
<td>76 mL/min/kg</td>
</tr>
</tbody>
</table>

Bi-directional transport of 1 µM Compound 13 was measured in MDR1-transfected LLC-PK1 cell monolayers; A-apical side; B-basolateral side. PPB was determined by equilibrium dialysis in CD-1 mouse plasma with 1 µM Compound 13. Hepatic CL was predicted based on the t\text{1/2} of 1 µM Compound 13 in CD-1 mouse microsomes (1 mg/mL) or hepatocytes (1x10^6 cells/mL).

Table 4. PK of Compound 13 in CD-1 Mice

<table>
<thead>
<tr>
<th>Dose</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/kg IV</td>
<td>CL</td>
<td>46 mL/min/kg</td>
</tr>
<tr>
<td></td>
<td>t\text{1/2}</td>
<td>0.96 hr</td>
</tr>
<tr>
<td></td>
<td>V\text{ss}</td>
<td>0.53 L/kg</td>
</tr>
<tr>
<td>100 mg/kg PO</td>
<td>C\text{max}</td>
<td>0.59 µg/mL</td>
</tr>
<tr>
<td></td>
<td>T\text{max}</td>
<td>1.00 hr</td>
</tr>
<tr>
<td></td>
<td>AUC\text{int}</td>
<td>0.88 hr^* µg/mL</td>
</tr>
</tbody>
</table>

CD-1 mice (n=3/time-point) were dosed IV with 3 mg/kg or PO with 100 mg/kg Compound 13 in 10% Captisol® and 50 mM sodium citrate, pH 5. Plasma was collected at 1, 5 (IV only), 15, 30 min, 1, 2, 4, 8, 12, 24 hr, and PK parameters were calculated on the mean concentration data using non-compartmental analysis.

Table 5. Compound 13 Concentrations After IP Dose in Mice

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Compound 13 Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.5 ± 1.3</td>
</tr>
<tr>
<td>1</td>
<td>0.97 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>BLQ</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
</tr>
<tr>
<td>15 mg/kg</td>
<td>26 ± 16</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>ND</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>13 ± 1.2</td>
</tr>
</tbody>
</table>

CD-1 mice (n=3/time-point) were dosed IP with 15, 50, or 100 mg/kg Compound 13 in 10% Captisol® and 50 mM sodium citrate, pH 5. Concentrations are reported as the arithmetic mean ± standard deviation. BLQ = below limit of quantitation. ND = not determined.

The KRAS-G12C mutant H358 cell line was used for in vitro screening of pERK modulation as the conditions for the assay were well developed. However, the MIA PaCa-2 line outperformed the H358 cell line in terms of tumor growth in nude mice and reproducibility of tumor doubling times. For those reasons tumor growth inhibition studies were conducted in the MIA PaCa-2 line. Similar to what was observed in H358 cells, the IC\text{50} of 13 was 48 nM in a pERK inhibition assay in MIA PaCa-2 cells. A tumor growth inhibition study was run with compound 13 administered IP QD in MIA PaCa-2 tumor bearing mice. The animals were randomized with 6 animals per group and dosing began on day 15 when the tumor volume had reached a size of 150 mm³. Tumor growth curves for vehicle control, the 30 mg/kg, and the 100 mg/kg doses are illustrated in Figure 3. The vehicle control tumors grew unabated, tripling in size by day 25. Both of the treated groups showed rapid tumor regressions after 5 days of dosing and then a leveling off of tumor volume with continued dosing. It should be noted that on day 25 there were 2 animals in the 30 mg/kg group and 4 animals in the 100 mg/kg group that were found to be tumor free. Compound 13 was well tolerated throughout this experiment and animals did not show weight loss or adverse symptoms.
Based on a published report by Patricelli, compound 13 target engagement was evaluated in MIA PaCa-2 tumor tissue from a mouse subcutaneous xenograft model. Mice were dosed IP QD for 5 days and tumors were harvested at 3 hr and 24 hr post dose on days 1 and 5. An LCMS-based KRAS-G12C engagement assay was developed to quantitatively measure the interaction of compound 13 with its intended protein target. The decrease of the cysteine 12 containing peptide from tryptic digests of KRAS-G12C mutant tumors following compound treatment was quantified relative to a control peptide, representing total KRAS. Following multiple doses of compound 13, KRAS-G12C engagement was maintained at >65% in tumors from both dose groups (Figure 4). Target engagement only slightly decreased between 3 hr and 24 hr, despite a large decrease in concentration of compound 13 in the plasma (Table 5), supporting a sustained target residence time and indicating a relatively slow rate of KRAS-G12C protein synthesis. Coupled with the tumor growth inhibition results, these data show on-target efficacy for the tetrahydropyridopyrimidine KRAS-G12C inhibitor 13.

In summary, we have identified a series of tetrahydropyridopyrimidines as irreversible covalent inhibitors of KRAS-G12C. A crystal structure of prototype compound 4 was used to optimize the interactions with the protein for enhanced potency. Replacement of the napthyl with a naphthol and substitution at C-2 of the pyrimidine ring resulted in compound 13 possessing a cell IC50 = 70 nM. Tumor regressions and cures were observed in a TGI study with compound 13 dosed IP QD at 30 and 100 mg/kg. In addition, an on-target mechanism of action was confirmed via KRAS-G12C target engagement experiments. Compound 13 is an advanced lead; future publications will detail our findings toward potent, orally bioavailable compounds with enhanced PK.

ASSOCIATED CONTENT

Coordinates for the X-ray structures shown in Figures 1 and 2 will be deposited with the Protein Data Bank.

AUTHOR INFORMATION

Corresponding Author
* Phone 303-386-1528, Email brad.fell@arraybiopharma.com

Author Contributions
The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

We thank the in vitro ADME group at Array BioPharma Inc.

ABBREVIATIONS

ADME, absorption, distribution, metabolism, and excretion; AUC0-inf, area under the curve extrapolated to infinity; BLQ, below the lower limit of quantitation; CBZ, carboxybenzyl, CL, clearance; Cmax, maximum concentration; DCM, dichloromethane; DMA, dimethylacetamide; F, bioavailability; LCMS, liquid chromatography mass spectrometry; POC, percent of control; ND, not detected; PD, pharmacodynamics; P-gp, P-glycoprotein; PK, pharmacokinetics; PPB, plasma protein binding; Tmax time at Cmax; TFA, trifluoroacetic acid; TGI, tumor growth inhibition;

REFERENCES


10. KRAS-G12C lite has all cysteines except G12C mutated to other amino acids (C51S/C80L/C118S), as in Ostrem et al.¹


学霸图书馆
www.xuebalib.com

本文献由 “学霸图书馆-文献云下载” 收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务” 的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页  文献云下载  图书馆入口  外文数据库大全  疑难文献辅助工具