2-Formylpyridyl Ureas as Highly Selective Reversible-Covalent Inhibitors of Fibroblast Growth Factor Receptor 4

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Supporting Information

ABSTRACT: As part of a project to identify FGFR4 selective inhibitors, scaffold morphing of a 2-formylquinoline amide hit identified series of 2-formylpyridine ureas (2-FPUs) with improved potency and physicochemical properties. In particular, tetrahydronaphthyridine urea analogues with cellular activities below 30 nM have been identified. Consistent with the hypothesized reversible-covalent mechanism of inhibition, the 2-FPUs exhibited slow binding kinetics, and the aldehyde, as the putative electrophile, could be demonstrated to be a key structural element for activity.

KEYWORDS: 2-Formylpyridines, fibroblast growth factor receptor 4, kinase inhibitors, reversible-covalent binders, binding kinetics, hepatocellular carcinoma

Fibroblast growth factor 4 (FGFR4) is one of a family of four receptor tyrosine kinases, which are regulated by their interactions with a much larger number of fibroblast growth factors (FGFs). FGFR signaling is involved in the control of multiple developmental and physiological processes, as well as being implicated as a driver in certain forms of cancer.¹ A number of pan-FGFR inhibitors are currently undergoing clinical investigation for the treatment of a range of FGFR 1–3 driven cancers.² More recently, FGF19 signaling through FGFR4, which also requires the presence of the coreceptor β -klotho, has been highlighted as a driver in a subset of solid tumors, in particular hepatocellular carcinoma (HCC).³ As a result, a number of groups have sought to identify selective inhibitors of FGFR4 signaling as a way to treat these cancers while avoiding the on-target side-effects associated with FGFR1–3 inhibition⁴ and, in particular, FGF23-mediated hyperphosphatemia.⁵ Selective agents have the potential to not only be better tolerated than pan-FGFR inhibitors but also to enable the impact of more robust inhibition of FGFR4 to be investigated.

To explore this possibility, we, and others, have identified a poorly conserved cysteine residue within the middle-hinge region of the FGFR4 ATP binding pocket that can be targeted to gain selectivity.⁴ Cys552 is situated two positions beyond the gate-keeper (GK+2) residue in FGFR4 and presents an opportunity for selectivity based upon the relatively small size of the side chain, or as a nucleophile for covalent binding. The other FGFR family members possess a tyrosine at the GK+2 position, and within the human kinome, only 12 other kinases have an equivalent sized, or smaller residue, at this position, of which only four contain a cysteine.⁶ Figure 1 shows the structures of selective FGFR4 inhibitors that have been described to date. Compounds 1 and 2 achieve FGFR4 selectivity by exploiting the

size difference in the middle-hinge region, and the acrylamides H3B-6527, BLU9931, BLU554, 3, and 4 by making a covalent bond with Cys552.⁴ In this Letter, we report the first part of our work, which ultimately led to the identification of FGF401, a highly selective inhibitor of FGFR4, which is currently undergoing clinical evaluation for the treatment of FGFR4 and β -klotho positive solid tumors.^{7–9}

We have previously described aspects of our FGFR4 inhibitor hit-finding activities, which included the identification of the 2-formylquinoline amide series, exemplified by 5, Figure 2, as a highly selective starting point.⁴ Before committing to an expansive optimization effort with this series, a number of key questions were raised. Of these, a greater understanding of the mechanism of FGFR4 inhibition was considered important to guide the optimization, in particular, exploring the role of the aldehyde, as the putative electrophile, reacting in a reversible-covalent manner through the formation of a hemithioacetal with Cys552 of FGFR4.¹⁰ However, the low solubility of 5 made in vitro testing problematic and hindered answering the above question. Therefore, identifying opportunities to improve physical properties, while also being compatible with improving the biological profile, became the first priority.

The poor solubility of 5 was apparent across a broad range of solvents, including aqueous media. As a result, in vitro pharmacology at concentrations of $>1 \mu\text{M}$ proved difficult to interpret with a high level of confidence. To overcome this limitation, one strategy was to reduce the coplanar aromatic nature of 5, which is reinforced by the intramolecular H-bond

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Figure 1. Selective inhibitors of the kinase activity of FGFR4.

Figure 2. Scaffold morphing to the 2-formyl THNU series 6.

between amide N–H and quinoline nitrogen. However, the intramolecular H-bond was also believed to be an important conformational restraint for FGFR4 activity.⁴ The introduction of sp^3 -hybridized atoms into the quinoline moiety was explored as a way to reduce the molecules coplanar aromatic nature, and as a result, the efficiency of intermolecular interactions within the solid state.¹¹ Morphing the quinoline amide moiety to the corresponding tetrahydronaphthyridine urea (THNU) generated the analogue 6a. The melting point for 6a (133 °C) was found to be lowered by 42 °C when compared to 5 (175 °C), consistent with the above hypothesis.¹² Although high throughput equilibrium solubility (HTES) remained outside the measured range ($<4 \mu\text{M}$),¹² more precise measurements indicated the thermodynamic solubility of 6a to be improved relative to 5 (aqueous pH 6.8 solubilities: 5 $< 2.9 \mu\text{M}$ and 6a = $4.9 \mu\text{M}$). More importantly, consistent results could be obtained with 6a in *in vitro* assays, with no evidence for artifacts. In addition, a four-fold increase in biochemical potency was observed with the THNU modification, Table 1, further supporting a scaffold morphing strategy in this direction.

The THNU analogue 6a was anticipated to bind within the ATP-pocket of FGFR4 in an analogous manner to that hypothesized previously for 5.⁴ Reaction between the Cys552 thiol and the aldehyde of 6a leads to the formation of the hemithioacetal adduct, shown in Figure 3, which is supported by the aminopyridyl moiety hydrogen bonding to the hinge residues

Table 1. Biochemical and Cellular FGFR4 Data^a

compd	IC ₅₀ (nM)	
	biochemical FGFR4	cellular Ba/F3 pFGFR4
1	2.2 ± 0.9	162 ± 52
BLU9931	14 ± 10	
5	57 ± 10	2755 ± 177
6a	13 ± 9	
6b	16 ± 5	108 ± 33
6c	21	916 ± 657
6d	300	
6e	6.3	72 ± 16
6f	0.7	29
6g	1.3 ± 0.5	18 ± 8
6h	11 ± 5	88 ± 8
6i	17	150 ± 112
6j	27	224
6k	1.2	24
7a	45 ± 8	131 ± 24
7b	33 ± 4	
7c	5200	
7d	9500	
7e	790	>3000
7f	23 ± 17	2800 ± 283
7g	140	>3000
8	>10000	
9	42 ± 14	531 ± 57
10a	3.2 ± 1.1	750 ± 136
10b	1.1 ± 0.4	55 ± 3
11a	660 ± 57	>3000
11b	175 ± 21	741 ± 77
12a	6.5 ± 2.5	41 ± 35
12b	7.4 ± 2.0	91 ± 23
13	>10000	
14	4400	

^aData are from single experiments, or expressed as mean ± SD, where 2 to 5 repeated experiments were performed.

Figure 3. Model of 6a bound within the ATP binding site of FGFR4. The compound forms hydrogen bonds with residues Ala553 and Val500 and a pseudo hydrogen bond with Glu551, in addition to the intramolecular hydrogen bond. Hydrogen bonds are indicated by the dotted violet lines.

Glu551 and Ala553. Based upon this model, the opportunity to further increase potency, and to regulate physical properties, was anticipated to be possible through the modification of the hinge binding element within 6a.

Figure 4. Hinge-binding variants 7a–g prepared in the 2-formyl THNU series.

As a first step toward exploring the hinge-binding structure–activity relationship (SAR) surrounding 6a, a number of alternative heterocyclic groups were evaluated, Figure 4. Of these, only the 2-pyrimidinyl and 2-pyrazinyl derivatives 7a and 7b and the extended 2-pyrazolyl derivative 7f led to activities approaching the 2-pyridyl analogues. For 7f, the 3,5-dimethoxyphenyl moiety is anticipated to make the key back-pocket interactions described previously for several FGFR inhibitors.¹³ The 2-pyridyl hinge-binder was taken for further exploring the SAR, in particular through substitution at the 4- and 5-pyridyl positions based upon the modeled interaction shown in Figure 3. In the 5-pyridyl position (R^1 , Figure 5), trifluoromethyl 6a, cyano

Compound	R^1	R^2
6a	CF ₃	H
6b	CN	H
6c	Cl	H
6d	H	H
6e	CN	OMe
6f	CN	O ^t Pr
6g	CN	OCH ₂ CH ₂ OMe
6h	CN	OCH ₂ CH ₂ NMe ₂
6i	CN	--C ₄ H ₈ O
6j	CN	--N ₂ H ₄ O
6k	CN	--N ₂ H ₄ OH

Figure 5. Structures of the THNU analogues 6a–k.

6b, and chloro 6c all increased the potency to a similar extent when compared to the unsubstituted pyridine ring 6d. The cyano group in the 5-position was of particular interest based upon the lower lipophilicity (logD_{7.4} 6a, 4.1; 6b, 2.9; 6c, 3.7).¹² In the 4-pyridyl position (R^2 , Figure 5), a range of C-, N-, and O-substituents 6e–k, were designed to fill a small hydrophobic cleft, formed between Gly474 and Val481, and to make van der Waals contacts with Leu619.¹² Encouragingly these modifications were found to further increase potency. In particular, the larger 5-pyridyl ether analogues 6f and 6g and the 5-pyridyl N-(4-hydroxy-4-methyl)-piperidinyl analogue 6k resulted in FGFR4

biochemical activities below 2 nM. These interactions are highlighted for 6k in Figure 6.

Figure 6. Model of 6k bound within the FGFR4 ATP pocket highlighting the hydrophobic interactions of the R^2 group.

The introduction of a basic amine in 6h led to an improvement in aqueous solubility (HTES 300 μ M), albeit with a slight reduction in potency. In addition, high permeabilities were determined for all the compounds and were consistent with the potential for high levels of absorption from the gut following oral administration.¹² Taken together, these data supported the hypothesized binding mode and indicated that the potency and physicochemical properties of the series could be modulated in a favorable way through modification of the hinge-binding moiety.

Based upon the improvements derived from switching the quinoline amide to the THNU scaffold, further 2-formylpyridine urea (2-FPU) variants were prepared to explore the SAR of the core-structure linking the hinge binder to the aldehyde electrophile, Figure 7. In each case, the new scaffold morphs were designed to preserve the intramolecular hydrogen-bond, which was considered to be essential for the optimal alignment of the two key structural elements. Support for this was derived from the FGFR4-inactive N-methylated analogue 8, Figure 8, in which the intramolecular hydrogen-bond is absent, and the required electrophile/hinge-binding alignment is highly disfavored.¹² These data also indicate that the aldehyde functionality alone is not sufficient for strong FGFR4 inhibitory activity.

Introduction of oxygen into the saturated THN ring gave the analogue 9, which showed a three-fold decrease in FGFR4 activity. Reducing the size of the saturated THN to the five-membered analogues retained a similar level of activity for both the aromatic 10a and dihydro 10b analogues. However, although isolatable, the pyrrolopyridine urea 10a was very susceptible to hydrolysis and was not considered to be a viable option for further evaluation. Increasing the ring size to the seven-membered analogues 11a and 11b led to a >50-fold decrease in potency. This decrease in activity was rationalized by modeling to arise from an unfavorable interaction with a hydrophobic slot, formed between residues Leu473 and Gly556, at the solvent

Figure 7. 2-FPU analogues 9–12 prepared as scaffold morphs of the quinoline-amide hit 5.

Figure 8. Structures of the *N*-methyl urea 8, benzylic alcohol 13, and desformyl analogue 14.

exposed side of the FGFR4 ATP binding site.¹² This slot was considered too narrow to readily accommodate the larger ring size.

Due to the presence of the intramolecular hydrogen-bond in the above compounds, they can be considered to be pseudo tricyclic structures. Therefore, the opening up of the saturated ring of the THN was not anticipated to impact upon the overall alignment of the key structural elements. Consistent with this analysis, the ring opened analogues 12a and 12b retained a similar level of potency to the THN starting point 6. Additionally, modeling highlighted that a range of urea *N*-substituents would have the potential to make favorable interactions,¹² in particular, within the hydrophobic slot formed between Leu473 and Gly556, described above, not only providing the opportunity to improve the FGFR4 activity but also to further regulate physicochemical properties (HTES 12b > 1 mM).

Assessing FGFR family selectivity, 2-FPUs 5–12 all exhibited excellent selectivity versus FGFR2 and when measured versus FGFR1 and FGFR3.¹² Additionally, selectivity against a diverse panel of 52 kinases was excellent, the majority of kinases tested exhibited IC₅₀ values >10 μM, with no IC₅₀ values <1 μM.¹² As described previously for 5, biochemical activity was completely lost using a variant of the FGFR4 kinase domain in which Cys552 was exchanged for alanine, indicating the reversible-covalent interaction remains valid for the 2-FPU compounds.^{4,12}

Cellular activity was assessed using a Ba/F3 cell model in which the FGFR4 kinase domain is constitutively activated by fusion with the translocation ets leukemia transcription factor (TEL). Inhibition of FGFR4 autophosphorylation was measured following a 1 h incubation with the test compounds, Table 1.¹² For the majority of the 2-FPU analogues, a consistent five- to 50-fold IC₅₀ shift from the biochemical to cellular assay was observed, which is slightly lower than the 74-fold shift observed for the noncovalent FGFR4-selective reference compound 1. Exceptions being the pyrrolopyridine urea 10a and extended 2-pyrazoyl derivative 7f where greater than 100-fold shifts were observed. For 10a, this is likely a result of the chemical instability associated with the urea linkage described above. Overall, the Ba/F3 cellular assay data showed the potential of the THNU series, with three analogues displaying IC₅₀ values below 30 nM.

With potent, soluble, and highly selective compounds identified, we next sought to investigate further the mechanism of FGFR4 inhibition. One consequence of the proposed reversible-covalent interaction was anticipated to be extended binding kinetics.¹⁴ To gain an understanding of the inhibitor/FGFR4 interaction kinetics, competition binding experiments were conducted with three of the 2-FPU analogues from Table 1.¹² All three 2-FPU analogues exhibited classical monoexponential binding, from which kinetic parameters could be derived, Table 2. In contrast, the noncovalent reference compound 1

Table 2. Binding Kinetic Data with the FGFR4 Kinase Domain

compd	K _d (nM)	k _{on} (M ⁻¹ min ⁻¹)	k _{off} (min ⁻¹)	residence time (min)
1 ^a	20			<1.4
6a	28	2.2 × 10 ³	3.7 × 10 ⁻³	272
6b	5.0	1.4 × 10 ⁴	4.3 × 10 ⁻³	235
9	35	4.5 × 10 ³	9.6 × 10 ⁻³	105

^aBinding kinetics are too fast to measure, and the residence time is below the measurable lower limit for the assay of 1.4 min.

dissociated from the FGFR4 kinase domain faster than the labeled probe used in the assay, and as a result, no kinetic parameters could be assigned. The 2-FPU analogues exhibited slow-binding kinetics, with 1.5–4.5 h residence times, that are at least 75× longer than for 1, while falling within a similar affinity range. Interestingly, inhibitors possessing longer target residence times have been associated with enhanced selectivity and higher levels of in vivo activity.¹⁵

Having determined slow binding kinetics with the 2-FPUs, the biochemical assay was evaluated to understand which conditions would reflect the maximum equilibrium IC₅₀ values. The approach taken was analogous to that which has been used to characterize a number of slow-binding type-II kinase inhibitors.¹⁶ Starting with a nonphosphorylated FGFR4 kinase domain construct, the inhibitor preincubation times were sequentially increased from 0 to 4 h prior to initiating the phosphorylation reaction (1 h incubation).¹² No change up to a modest five-fold increase in potency was observed when the preincubation period was increased to 1 h. However, no further increases were noted with longer preincubations for the majority of compounds.

The biochemical data shown in Table 1 were generated using the “standard” screening conditions with a longer FGFR4 kinase-domain construct of mixed phosphorylation states and were derived without preincubation (1 h phosphorylation reaction).⁴ Assuming the same temporal relationship to the data in Table 2: the biochemical data with the “standard” assay in Table 1 would then also be anticipated to be within five-fold of the maximum equilibrium IC₅₀ values, for the majority of the 2-FPUs. Therefore, with the possibility for only a small IC₅₀ shift, we positioned the “standard” biochemical assay as the primary

measure of FGFR4 activity going forward and planned only to spot check the binding kinetics for key compounds to monitor for any significant change in profile.

The limiting kinetic factor in HCC was anticipated to be the relatively rapid FGFR4 resynthesis rate (<2 h),⁴ suggesting no obvious benefit for inhibitors with residence times beyond this value. In fact, a faster target engagement was anticipated to be more advantageous in this situation, to rapidly engage the newly synthesized protein and to ensure the maximum level of inhibition can be maintained without the need for high inhibitor concentrations. This target kinetic understanding was pivotal at the onset of the project to prioritize the 2-FPU series ahead of other covalent hit series interacting by what would be considered irreversible-covalent interactions and, ultimately, differentiated the project from the acrylamide-based inhibitors described by other groups.⁴ With such a rapid target turnover, and the need for a continuous high level of pathway inhibition to achieve maximum efficacy, sustained exposure to a reversible-covalent inhibitor was considered to be safer and to potentially offer a simpler pharmacokinetic opportunity.⁴

The slow-binding kinetics provided further support for the hypothesized reversible-covalent interaction for the 2-FPU series with FGFR4. However, we sought additional evidence for this mechanism of action as a way to increase our understanding of the inhibitor–protein interaction and to assist with the further optimization of the series. Consistent with our previous observations, the benzylic alcohols derived by reduction of the aldehyde, as exemplified by **13** in Figure 8, showed no FGFR4 inhibitory activity.⁴ However, in the absence of covalent binding, an unfavorable steric interaction was anticipated to occur between the side chain of Cys552 and the benzylic alcohol of **13**.¹² Therefore, to better understand the strength of the hinge-binding interaction and the contribution of the aldehyde, the desformyl analogue of the more soluble **6h** was prepared, compound **14** in Figure 8 (HTES, 173 μ M). This compound inhibited FGFR4 with a biochemical activity 400 $\times$ weaker than the parent **6h** and highlighted the key contribution from the aldehyde, consistent with the proposed mechanism of action. Interestingly, this makes for a very different situation for a number of acrylamide-based irreversible-covalent kinase inhibitors, where the electrophile has typically been introduced into an already low nanomolar hinge-binding scaffold.¹⁷

When preparing the 2-FPU derivatives, a number of commonly applied approaches for forming the central urea-linkage were found to be inefficient, which was attributed to the low nucleophilicity of the 2-aminopyridines. Two methods that were discovered to reliably provide access to a wide range of bis-aminopyridyl ureas in good yield are exemplified by the syntheses of **6b** and **11a**, in Schemes 1 and 2.

Preparation of **6b** started with the acylation of *N*-lithiated 2-(dimethoxymethyl)-tetrahydro-naphthyridine **15** with diphenyl carbonate to prepare the corresponding phenyl carbamate

Scheme 1. Synthesis of the THNU Analogue **6b**^a

^aReagents and conditions: (a) LiHMDS, (PhO)₂CO, THF, −15 °C, 1.5 h, 78%; (b) LiHMDS, 2-amino-5-cyanopyridine, THF, −15 °C, 1.5 h, 94%; (c) HCl(aq), THF, 25 °C, 1 h, >95%.

Scheme 2. Synthesis of the 2-FPU Analogue **11a**^a

^aReagents and conditions: (a) PhOC(O)Cl, pyridine, THF, 25 °C, 5 h, 84%; (b) **19**, DMAP, THF, reflux, 23 h, 54%; (c) HCl(aq), THF, 25 °C, 1 h, >95%.

intermediate **16**. Further reaction of **16** with *N*-lithiated 2-amino-5-cyanopyridine **17** formed the urea, and subsequent acid hydrolysis of the acetal gave **6b** in 70% overall yield. To prepare **11a**, the phenyl carbamate of the 2-aminopyridine **18** was heated with the 2-(dimethoxymethyl)-dihydroazepine **19** in a reaction that likely proceeds via the highly reactive and labile 2-pyridylisocyanate.¹⁸ The acetal group in the resulting urea was then hydrolyzed to give **11a** in 43% overall yield.

In conclusion, scaffold morphing from a quinolone-amide hit identified a number of 2-FPU variants with increased FGFR4 activity and improved physicochemical properties. In particular, potent and selective THNU **6**, dihydropyrrolopyridine urea **10**, and ring-opened urea **12** analogues were identified for further optimization, with examples of cellular FGFR4 activities below 50 nM. Characterization of the binding kinetics and preliminary SAR surrounding the 2-FPUs remained consistent with the hypothesized reversible-covalent interaction. This, in combination with an understanding of the FGFR4 turnover rate in HCC, provided insights into how best to evaluate and optimize the series going forward. Future disclosures will focus on how the leads and mechanistic understanding, described herein, were used to identify the clinical candidate FGF401.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00485.

Synthesis of compounds and characterizing data; physicochemical measurements; biochemical and cellular kinase assays (PDF)

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Notes

The authors declare the following competing financial interest(s): Authors include employees of Novartis AG and/or stockholders of Novartis AG.

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■ ABBREVIATIONS

FGFR4, fibroblast growth receptor 4; FGFs, fibroblast growth factors; HCC, hepatocellular carcinoma; GK+2, gate-keeper residue plus two; THNU, tetrahydronaphthyridine urea; HTES, high throughput equilibrium solubility; 2-FPU, 2-formylpyridine urea; SAR, structure–activity relationship; TEL, translocation ets leukemia; LiHMDS, lithium hexamethyldisilazide; DMAP, 2,6-dimethylaminopyridine.

■ REFERENCES

- (1) Carter, E. P.; Fearon, A. E.; Grose, R. P. Careless talk cost lives: fibroblast growth factor signaling and the consequences of pathway malfunction. *Trends Cell Biol.* **2015**, *25*, 221–233.
- (2) Lemieux, S.; Hadden, M. K. Targeting the fibroblast growth factor receptors for the treatment of cancer. *Anti-Cancer Agents Med. Chem.* **2013**, *13*, 748–761.
- (3) Gauglhofer, C.; Paur, J.; Schrottmaier, W. C.; Wingelhofer, B.; Huber, D.; Naegelen, I.; Pirker, C.; Mohr, T.; Heinzle, C.; Holzmann, K.; Marian, B.; Schulte-Hermann, R.; Berger, W.; Krupitza, G.; Grusch, M.; Grasl-Kraupp, B. Fibroblast growth factor receptor 4: a putative key driver for the aggressive phenotype of hepatocellular carcinoma. *Carcinogenesis* **2014**, *35*, 2331–2338.
- (4) Fairhurst, R. A.; Knoepfel, T.; Leblanc, C.; Buschmann, N.; Gaul, C.; Blank, J.; Galuba, I.; Trappe, J.; Zou, C.; Voshol, J.; Genick, C.; Brunet-Lefevre, P.; Bitsch, F.; Graus-Porta, D.; Furet, P. Approaches to selective fibroblast growth factor receptor 4 inhibition through targeting the ATP-pocket middle-hinge region. *MedChemComm* **2017**, *8*, 1604–1613.
- (5) Hierro, C.; Rodon, J.; Tabernero, J. Fibroblast growth factor (FGF) receptor/FGF inhibitors: novel targets and strategies for optimization of response of solid tumors. *Semin. Oncol.* **2015**, *42*, 801–819.
- (6) Leproult, E.; Barluenga, S.; Moras, D.; Wurtz, J.-M.; Winssinger, N. Cysteine mapping in conformationally distinct kinase nucleotide binding sites: application to the design of selective covalent inhibitors. *J. Med. Chem.* **2011**, *54*, 1347–1355.
- (7) Fairhurst, R. A.; Knoepfel, T.; Furet, P.; Buschmann, N.; Leblanc, C.; Mah, R.; Kiffe, M.; Graus-Porta, D.; Weiss, A.; Kinyamu-Akunda, J.; Wartmann, M.; Trappe, J.; Gabriel, T.; Hofmann, F.; Sellers, W. R. FGF401: A reversible-covalent inhibitor of FGFR4 for the treatment of hepatocellular carcinoma. Presented at the 253rd National Meeting, American Chemical Society, San Francisco, CA, MEDI 353, April 5th, 2017.
- (8) Graus-Porta, D.; Weiss, A.; Stamm, C.; Fairhurst, R. A.; Wartmann, M.; Knoepfel, T.; Leblanc, C.; Buschmann, N.; Blank, J.; Furet, P.; Galuba, I.; Trappe, J.; Mignault, A.; Ramkumar, T.; Kauffmann, A.; Engelman, J. A.; Hofmann, F.; Sellers, W. R. NVP-FGF401 a first-in-class highly selective and potent FGFR4 inhibitor for the treatment of HCC. Presented at the AACR Annual Meeting, American Association for Cancer Research, Washington, April 1–5, 2017; abstract 2098.
- (9) Weiss, A.; Graus-Porta, D.; Reimann, F.; Buhles, A.; Stamm, C.; Fairhurst, R. A.; Kinyamu-Akunda, J.; Sterker, D.; Kiffe, M.; Murakami, M.; Wartmann, M.; Wang, Y.; Engelman, J. A.; Hofmann, F.; Sellers, W. R. NVP-FGF401: Cellular and in vivo profile of a novel highly potent and selective FGFR4 inhibitor for the treatment of FGF19/FGFR4/KLB+ tumors. Presented at the AACR Annual Meeting, American Association for Cancer Research, Washington, April 1–5, 2017; abstract 2103.
- (10) Caraballo, R.; Dong, H.; Ribeiro, J. P.; Jiménez-Barbero, J.; Ramström, O. Direct STD NMR identification of β -galactose inhibitors from a virtual dynamic hemithioacetal system. *Angew. Chem., Int. Ed.* **2010**, *49*, 589–593.
- (11) Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009**, *52*, 6752–6756.
- (12) Further details are included in the [Supporting Information](#).
- (13) Tucker, J. A.; Klein, T.; Breed, J.; Overman, R.; Phillips, C.; Norman, R. A. Structural insights into FGFR kinase isoform selectivity: diverse binding modes of AZD4547 and ponatinib in complexes with FGFR1 and FGFR4. *Structure* **2014**, *22*, 1764–1774.
- (14) Bernetti, M.; Cavalli, A.; Mollica, L. Protein-ligand (un)binding kinetics as a new paradigm for drug discovery at the crossroad between experiments and modelling. *MedChemComm* **2017**, *8*, 534–550.
- (15) Copeland, R. A. The drug-target residence time model: a 10-year retrospective. *Nat. Rev. Drug Discovery* **2016**, *15*, 87–95.
- (16) Grütter, C.; Simard, J. R.; Mayer-Wrangowski, S. C.; Schreier, P. H.; Pérez-Martín, J.; Richters, A.; Getlik, M.; Gutbrod, O.; Braun, C. A.; Beck, M. E.; Rauh, D. Targeting GSK3 from *ustilago maydis*: type-II kinase inhibitors as potential antifungals. *ACS Chem. Biol.* **2012**, *7*, 1257–1267.
- (17) Wang, Y.; Chen, Z.; Dai, M.; Sun, P.; Wang, C.; Gao, Y.; Zhao, H.; Zeng, W.; Shen, L.; Mao, W.; Wang, T.; Hu, G.; Li, J.; Chen, S.; Long, C.; Chen, X.; Liu, J.; Zhang, Y. Discovery and optimization of selective FGFR4 inhibitors via scaffold hopping. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2420–2423.
- (18) Holt, J.; Andreassen, T.; Bakke, J. M.; Fiksdahl, A. Nitropyridyl isocyanates. *J. Heterocycl. Chem.* **2005**, *42*, 259–264.