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Aryl extensions of thienopyrimidinones as fibroblast growth factor receptor 1 kinase inhibitors

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This paper is dedicated with great affection to Prof. Harry H. Wasserman, friend and Yale colleague, on the occasion of his 90th birthday

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ABSTRACT

Optimization of thienopyrimidinone derivatives as FGFR1 kinase inhibitors is being pursued. The present results confirm predictions of computational modeling that an aryl substituent can be introduced at the 2-position in structure **3**. The substituent is anticipated to project deeper into the binding site and provide opportunities for enhanced activity and selectivity. The most potent analog reported herein, **13**, has a 4-hydroxyphenyl substituent and yields an IC_{50} of 6 μM for inhibition of phosphorylation by FGFR1 kinase. It was also found that the western anisole-containing substituent in **3** can be replaced by a propionic acid group with no loss in potency and with potentially significant gains in pharmacologically relevant properties.

Fibroblast growth factors (FGF) play an important role in many biological processes including cell proliferation, migration, differentiation, survival, morphogenesis, and angiogenesis.[1] The diverse biological effects of FGFs are mediated by cell surface receptors (FGFR1-4) with intrinsic protein tyrosine kinase activity.[2] FGFR is activated by binding of FGF to its extracellular domain, resulting in receptor dimerization and autophosphorylation of specific tyrosine residues in the cytoplasmic domain. The activated FGFR interacts with downstream intracellular proteins to initiate signal transduction, such as in the MAPK and PKB/Akt pathways.[3] Abnormal FGF signaling either through gain or loss of function by mutations in FGF or FGFR has been implicated in cancer and other disorders, including skeletal, cardiovascular, immunological, and neurological diseases.[1,4] Therefore, targeted inhibition of FGFR kinases with ATP-competitive small molecule inhibitors has become an attractive therapeutic strategy. Several classes of such inhibitors have been reported, including indolinones,[5] pyrido[2,3-*d*]pyrimidines,[6] napthyridines,[7] triazines,[8] indenes,[9] quinolines,[10] and thioindoles.[11] However, only a limited number of FGFR inhibitors have entered clinical trials.[12–17] Furthermore, since the human genome encodes at least 518 protein kinases and nearly all kinase inhibitors target the well-conserved ATP binding site,[18] development of selective kinase inhibitors continues to be a significant challenge.

In the course of studies to identify new FGFR1 kinase inhibitors, we discovered two inhibitors **1** and **2**, a benzylidene derivative of pseudothiohydantoin and a thienopyrimidinone derivative, by structure-based virtual screening followed by initial lead optimization.[19] **1** and **2** inhibit phosphorylation by FGFR1 kinase with IC_{50} values of 23 and 1.9 μM, respectively. The pseudothiohydantoin **1** was also found to be an inhibitor of EGFR, Src, and InsR kinases with IC_{50} values of 10–56 μM. The thienopyrimidinone **2** was found to inhibit EGFR and Src kinases with IC_{50} values of 2.4 and 1.9 μM, while it did not inhibit InsR. Thus, little selectivity was observed with these compounds. In an effort to develop more potent and selective FGFR1 kinase inhibitors, the thienopyrimidinones were chosen for further optimization.

A structure from docking calculations[19] for the 21-μM inhibitor **3** complexed with FGFR1 kinase is illustrated in Figure 2A. Formation of two hydrogen bonds between the amide fragment in the pyrimidinone ring of **3** and Ala564 in the hinge region is anticipated. The tricyclic core of thienopyrimidinone **3** is spatially limited to the ATP binding site, and the western end of the inhibitor is solvent exposed. Exploration eastward, deeper into the active site, is expected to be potentially more fruitful, especially

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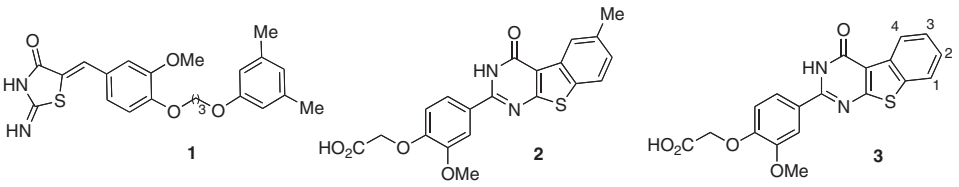


Figure 1. Chemical structure of **1**, **2**, and **3**.

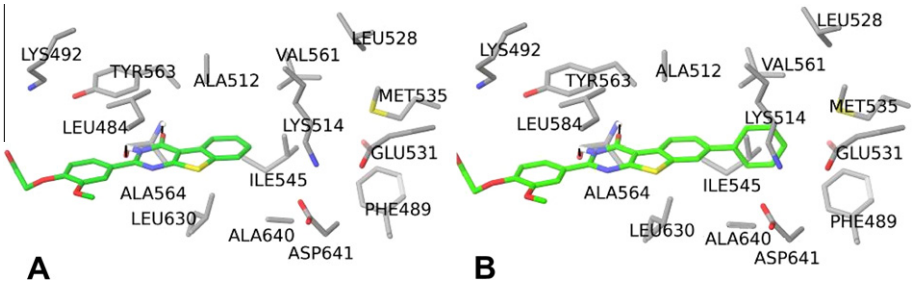
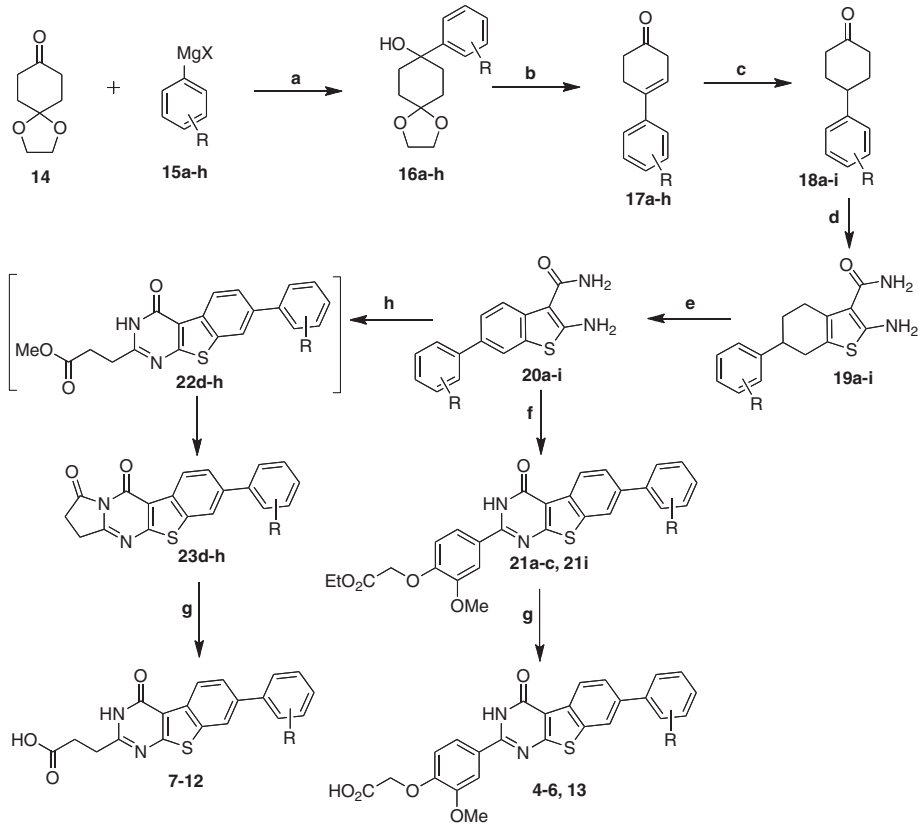


Figure 2. Computed structures of FGFR1 kinase with **3** (A) and **4** (B). Selected residues of FGFR1 kinase are shown; carbon atoms of the inhibitors are colored green. Hydrogen bonds are highlighted with black lines.



Scheme 1. Reagents and conditions: (a) RMgX, THF, 10 °C–rt, 3 h, 70–95%; (b) TFA, DCM, rt, 5 h, 84%-quant; (c) 10% Pd/C, EtOAc, rt, 16 h, 73–83%; (d) 2-Cyanoacetamide, S, piperidine, EtOH, 50 °C, 4 h, 42–50%; (e) *p*-chloranil, 1,4-dioxane, 90 °C, 5 h, 56–65%; (f) ethyl 2-(4-formyl-2-methoxyphenoxy)acetate (**24**), concd HCl, *n*-BuOH, reflux, 2 h, 57–80%; (g) 5 N NaOH, EtOH, reflux, 2 h, 60%-quant; (h) methyl-4-oxobutanone (**25**), concd HCl, *n*-BuOH, 80 °C, 8 h, 48–67%.

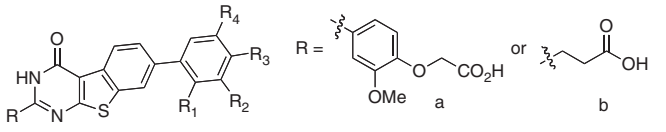
for selectivity owing to variation among kinases in this area. In the eastern end of **3** (see Figs. 1 and 2A), position 1 is solvent exposed and substitutions here should have limited effect on modulating activity. However, the de novo ligand-design program BOMB[20,21] (Biomolecular and Organic Molecule Builder) was used to consider introduction of substituents at positions 2–4 on the ligand in the binding site; the utilized protein coordinates came from the structure of the complex with a nicotinic acid derivative, which we previously reported (PDB ID: 3JS2).[19,22] Substituents larger than methyl cannot be introduced at positions 3 and 4 as they would

clash with the protein backbone and likely cause disruption of the critical hydrogen bonds between the tricyclic core and the hinge region. However, extension at the 2-position with monocyclic and possibly fused bicyclic aryl substituents emerged as feasible from the model building. The computed structure of the complex with **4** (Fig. 2B) shows that it appears possible to insert a phenyl group between Lys514 and Ile545 with potential benefits of cation–π interactions with Lys514 and hydrophobic contacts with Ile545, Val561, and Ala640. Importantly, this is achieved while retaining the position of the tricyclic core and the hydrogen bonds with the hinge region. The present Letter summarizes initial experimental results that have been obtained to test this prediction. Successful introduction of a phenyl group would open up exploration of a wide range of substituted analogs and other aryl substituents.

To pursue this notion, syntheses of compounds **4–13** with a phenyl group at the 2-position on the core of **3** were performed as summarized in Scheme 1. On the western end, **4–6** and **13** retain the substituted anisole ring as previously reported, for example, in **2**.[19] However, **7–12** incorporate a smaller alternative, propionic acid, that was found to not diminish activity (vide infra). Preparation of **5–12** commenced with ketal **14** and the syntheses of **4** and **13** began with commercially available cyclohexanones **18a** and **18i**. Grignard reaction of ketal **14** with phenyl magnesium halides **15a–h** produced hydroxy ketals **16a–h**. Simultaneous removal of the protecting group and dehydration with TFA provided **17a–h**, which after hydrogenation and Gewald reaction with 2-cyanoacetamide yielded 2-aminothiophenes **19a–i**. *p*-Choranil oxidation of **19a–i** delivered the benzothiophenes **20a–i**. Condensation of **20a–c** and **20i** with aldehyde **24** (see Scheme 1) resulted in esters **21a–c** and **21i**, which were hydrolyzed to give **4–6,** and **13**. Condensation of **20d–h** with aldehyde **25** afforded **23d–h** via intermediate formation of **22d–h**. Hydrolysis of esters **23d–h** produced **7–12**. The structures of **4–13** were validated through NMR and high-resolution mass spectrometry,[23] and their purities were typically >95% by HPLC. The ALPHAScreen assay was performed using purified FGFR1 kinase domain and a biotinylated peptide substrate, as described previously.[19] The assay results are summarized in Table 1.

The parent phenyl-containing analog **4** did show activity, 75 μM, though at a diminished level from **2** and **3**. Addition of a single methoxy group in the *meta* position, **5**, was found to lead to inactivity, while 3,5-dimethoxy substitution restored activity for **6** and **7** to about the level for **3**. Comparison of the results for **6** and **7** also confirms the point above that the two options for the westernmost part of the inhibitors are equally viable. Potential benefits of the change to the smaller propionic acid side chain are a predicted 100-fold enhancement in aqueous solubility in going from **6** to **7** and lowering of the computed log $P_{o/w}$ for **6** of 4.7 to 3.4 for **7**.[24] Mono substitution in the *meta* position was again found to lead to inactivity for the methyl and chloro analogs **9** and **12**. The one case with an *ortho* substituent, **10**, showed roughly a two-fold gain in activity over **4**. However, the methyl-scan represented by **8**–**10** revealed that *para*-substitution of the phenyl ring may be the most fruitful with the IC_{50} for **8** (26 μM) showing a three-fold improvement over the unsubstituted **4**. Additional gain was found for the *para*-chloro analog **11** at 18 μM, and finally the *para*-hydroxy analog **13** yielded an additional three-fold boost to 6 μM. Further examination of analogs with *ortho* and/or *para* substituents is indicated.

Table 1
Inhibitory activities of FGFR1 kinase by **4–13**



Compd	R	R_1	R_2	R_3	R_4	IC_{50}[a] (μM)
4	a	H	H	H	H	75
5	a	H	OMe	H	H	na
6	a	H	OMe	H	OMe	28
7	b	H	OMe	H	OMe	21
8	b	H	H	Me	H	26
9	b	H	Me	H	H	na
10	b	Me	H	H	H	43
11	b	H	H	Cl	H	18
12	b	H	Cl	H	H	na
13	a	H	H	OH	H	6

[a] na indicates not active in the assay.

In summary, as predicted by the computational modeling, aryl extension at the 2-position on the tricyclic core of theinopyrimidinone **3** was shown to be possible. It was also found that the anisole-based western substituent could be replaced by the smaller propionic acid alternative with no loss of activity and with concomitant expected enhancement of pharmacologically relevant properties. The most potent analog reported herein, the hydroxyphenyl analog **13**, yields inhibitory activity of 6 μM for FGFR1 kinase. Though the activity is similar to that of **2** (1.9 μM), the present results provide a platform for further elaboration of analogs to probe beyond the ATP binding site in search of enhanced interactions and selectivity. Such studies are underway with continued emphasis on the synergies between computational modeling, synthesis, and crystallography.

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23. *Spectral data for representative compounds*: 2-(4-(7-(3,5-dimethoxyphenyl)-4-oxo-3,4-dihydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-2-yl)-2-methoxyphenoxy) acetic acid (**6**) ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.50 (d, J = 8.3, 1H), 8.42 (d, J = 1.4 Hz, 1H), 7.90–7.82 (m, 3H), 7.03 (d, J = 9.3 Hz, 1H), 6.92 (d, J = 2.2 Hz, 2H), 6.53 (t, J = 2.2 Hz, 1H), 4.81 (s, 2H), 3.92 (s, 3H), 3.84 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.8, 168.3, 166.9, 160.9, 158.5, 154.4, 150.4, 148.6, 141.7, 137.8, 135.5, 133.0, 124.8, 124.1, 123.8, 121.4, 120.9, 115.1, 112.5, 111.3, 104.9, 99.7, 64.7, 55.7, 55.3; HRMS (ESI-TOF) calcd for $C_{27}H_{23}N_2O_7S$ [M+H]$^+$: 519.1226, found: 519.1221. 3-(7-(3,5-dimethoxyphenyl)-4-oxo-3,4-dihydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-2-yl)propanoic acid (**7**) ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.82 (s, 1H), 12.29 (s, 1H), 8.46 (d, J = 8.3 Hz, 1H), 8.40 (d, J = 1.4 Hz, 1H), 7.85 (dd, J = 1.7, 8.4 Hz, 1H), 6.91 (d, J = 2.2 Hz, 2H), 6.52 (t, J = 2.2 Hz, 1H), 3.83 (s, 6H), 2.96 (t, J = 6.9 Hz, 2H), 2.78 (t, J = 7.0 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.4, 166.6, 160.9, 159.7, 158.0, 141.7, 137.7, 135.2, 132.9, 124.8, 123.7, 120.9, 115.3, 104.9, 99.7, 55.3, 29.8, 28.9; HRMS (ESI-TOF) calcd for $C_{21}H_{19}N_2O_5S$ [M+H]$^+$: 411.1015, found: 411.1014.
24. Results from *QikProp* v. 3.0, Schrödinger Inc., New York, NY, 2006.