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Optimization of a series of 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidine inhibitors of IGF-1R: Elimination of an acid-mediated decomposition pathway

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ABSTRACT

Initial evaluation of a series 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidines revealed a C(1′) carboxamide was preferred for sub-micromolar in vitro potency against IGF-1R. Subsequent solution stability studies with **1** revealed a susceptibility toward acid-induced intramolecular cyclization with the C(1′) carboxamide. Herein, we describe several successful approaches toward generating both potent and acid-stable inhibitors of IGF-1R within the 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidine template.

The activation of IGF-1R in many human cancers coupled with the observation that inhibition of IGF-1R signaling results in a decreased proliferation of tumor cells in vitro and in vivo has made it an attractive oncology target.[1] As such, the optimization of inhibitors of IGF-1R has been reported by multiple groups in diverse chemical space.[2] We have previously disclosed the synthesis and optimization of a series of 4,6-bis-anilino-1*H*-pyrrolo[2,3-*d*]pyrimidines wherein a C(1′) carboxamide, which interacts with Asp1150 through a water-mediated hydrogen bond, was believed a prerequisite for potent inhibition of IGF-1R.[3,4]

Subsequent to our initial biological characterization of **1**, solution stability studies revealed a tendency toward decomposition in acidic media. Further investigation identified tetracycle **2** and acid **3** as the principle degradants (Scheme 1). Tetracycle **2** appeared to account for the unusual sensitivity of the carboxamide to mildly acidic media. In enzymatic estimates of IGF-1R potency, **2** and **3** showed reductions in potency relative to **1** of 20- and 4-fold, respectively. With the solution instability of **1** well-characterized, the design of potent analogs not suffering from this liability became a key medicinal chemistry aim.

The propensity toward hydrolysis did not prevent reasonable oral exposure of **1**, and as such allowed its use as an orally administered tool for ongoing drug-discovery efforts relating to IGF-1R. Isolation of authentic standards of **2** and **3** allowed their in vivo pharmacokinetics to be investigated. Tetracycle **2** exhibited slightly better exposure than parent **1** when dosed as a suspension in rat (Table 1), and accounted for about 10% of the total AUC when **1** was dosed as an oral suspension (Table 1). Carboxylic acid **3** showed no oral exposure, was rapidly cleared when dosed as an iv solution, and could not be detected in plasma following oral dosing of **1** or **2**. As a consequence of these data, medicinal chemistry efforts were focused on accessing potent analogs which would be more resistant toward in vivo hydrolysis in the stomach and especially in pharmaceutical formulations with pH <4.

Suspecting the electrophilicity of the carboxamide might correlate with decomposition rate (i.e., **1** → **2** → **3**), we measured the half-life for decomposition of parent (0.1 N HCl, 25 °C, LC–MS) as

Table 1
In vivo rat DMPK properties for **1**–**3** (data represents an average of three animals)

Compound	Dose (mg/kg)		Cl (mL/min/kg)	DNAUC (ng h/mL/mg/kg)			%*F* (parent)
	IV	PO		Parent	**2**	**3**	
1	2.1	9.2	51	156	18	0	65
2	2.2	9.8	47	203	–	0	81
3	1.4	9.8	>200	0	0	0	0

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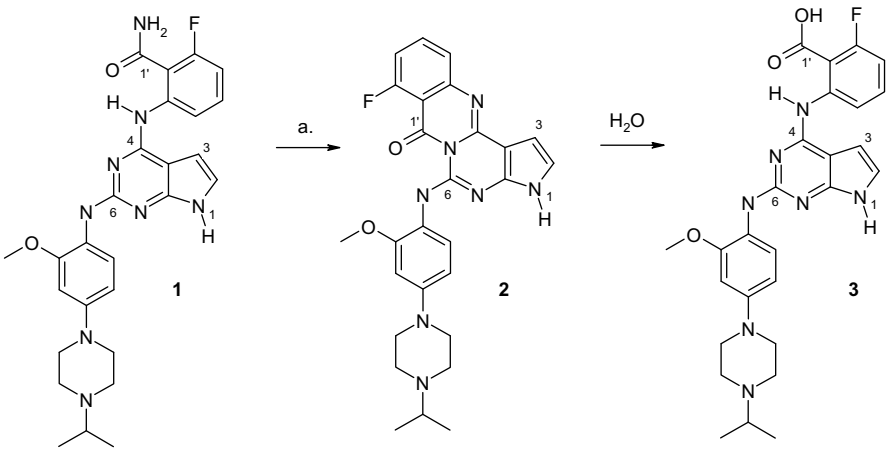
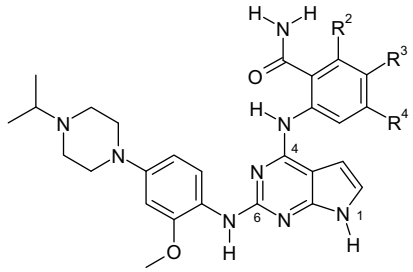


Scheme 1. Acid-induced intramolecular cyclization of **1**. Conditions: (a) 0.1 N HCl (aq), ambient temperature, $t_{1/2}$ = 17 h.

Table 2
Rates of decomposition (0.1 N HCl (aq) at 23 °C) and potency for substituted carboxamides



Compound	R^2	R^3	R^4	$t_{½}$ (h)	IGF-1R enzyme IC_{50} (nM)
1	F	H	H	17	2.0
4	H	F	H	2	0.9
5	H	H	F	4	0.7
6	F	F	H	17	1.3
7	F	H	F	39	0.8

Assay conditions used have been previously described and represent an average of ⩾2 independent measurements (see Ref. 4).

a function of substitution about the C(4) aniline (Table 2). Adding an electronegative fluorine at R^2 afforded half-lives 5- to 10-fold longer than identical substitution at R^3 or R^4. We hypothesized a hydrogen-bonding network including electronegative R^2 might account for the increased activation barrier toward hydrolysis for **1**, **6**, and **7**. Disubstitution (**7**) appeared to afford an additive impact on elongating $t_{1/2}$, but the overall impact of various substituents on R^2, R^3, and R^4 was subtle. All compounds (**1** and **4–7**) shared an unacceptable propensity ($t_{1/2}$ < 100 h) toward decomposition.

The most straightforward improvement in cyclization half-life would be anticipated to arise from removal of the offending carboxamide all-together. This proved a unique synthetic challenge, as the syntheses of **1** and **4–7** necessitated the design of an internal carboxamide-mediated activation strategy routed through the intermediacy of cyclized intermediates related to **22** (vide infra). This synthetic approach and its limitations has been described in detail elsewhere.[3] Ultimately we acknowledged that our chemistries for the preparation of **1** and **4–7** had evolved to rely on the very carboxamide functionality causing stability problems in acidic media. However, using a Buchwald–Hartwig approach,[5] we found we could prepare milligram quantities of a variety of analogs (Table 3) lacking a carboxamide in very modest yields. Of these analogs, dihydro-3*H*-indazole-3-one **11**, indoline **12**, and quinoline **13** retained reasonable potency at the enzyme level, but proved much less promising in the context of cellular estimates of IGF-1R inhibition (Table 3). Similarly, **9**, **10**, and **14–16** suffered from 10- to 100-fold losses in potency relative to **1** in both the IGF-1R enzyme and cellular assays. A suitably potent IGF-1R inhibitor lacking a carboxamide was not identified in the context of the isopropylpiperazine tail.

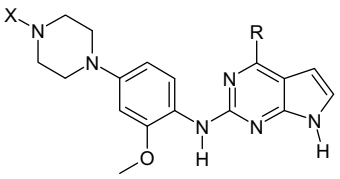
As we considered possible alternative strategies toward improving the stability within our series, a past synthetic problem piqued our interest. Specifically, we had found that although thiophene **17** added smoothly to key dichloro intermediate **8**, the subsequent formation of key, activated intermediate **21** and C(6) chloride displacement by aniline **19** does not occur to any appreciable extent upon exposure to protic or Lewis acids (i.e., **18** → **22**, Scheme 2), in direct contrast to the corresponding benzamides.[3] Although this unanticipated stability to acid was difficult to rationalize, we reasoned the same stability to acid that makes **18** an undesirable synthetic intermediate might ultimately impart acid stability on analogs related to it. We developed a modified synthetic protocol to allow access to heterocyclic **23–28**, which was routed through activated intermediate **21**, accessible via a non-acidic cyclization event (Scheme 2).

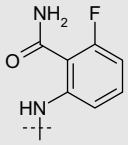
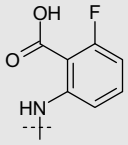
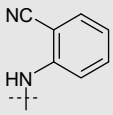
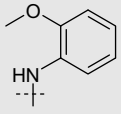
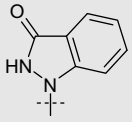
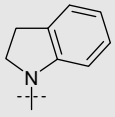
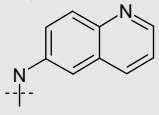
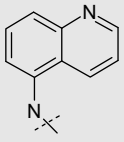
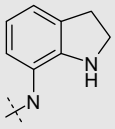
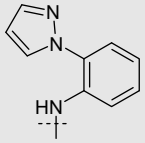
Acid-catalyzed addition of thiophene **20** to **8** was followed by base-mediated saponification of the methyl ester and exposure to oxalyl chloride to give key, activated intermediate **21**. Displacement of the C(6) chloride of isolated **21** with aniline **19** proceeded smoothly to afford **22**. Finally, ring-opening with ammonium hydroxide in THF/H_2O in a sealed tube followed by base-mediated hydrolysis of the tosylate protecting group afforded **23** in reasonable overall yield. This route allowed us to both bypass acid-stable but synthetically useless **18** and access primary carboxamides derived from amino-thiophenes as final analogs.

The reduced reactivity of **18** was found to correlate with desirable stability properties for **23–28**. Gratifyingly, **23–28** showed a tremendously reduced propensity toward acid-induced decomposition in 0.1 N HCl despite possessing a primary carboxamide. Additionally, no reduction in potency was observed via direct substitution of an amino-thiophene for the C(4) aniline. In fact **26–28** proved to be the most potent inhibitors of IGF-1R at both the cellular and enzyme level prepared during the course of our efforts (Table 4).

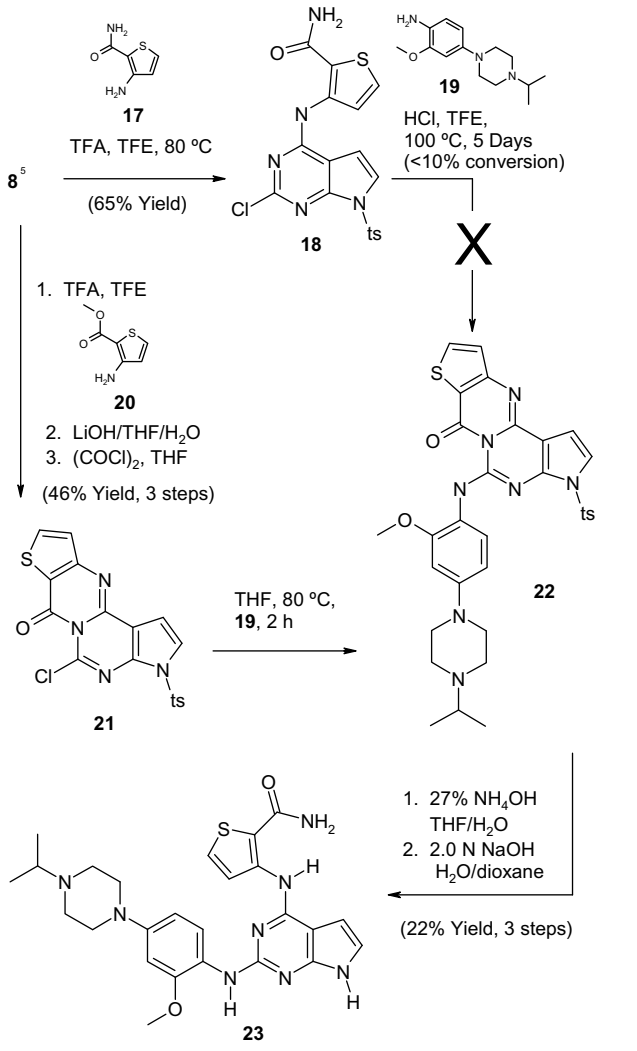
With the recognition that a C(4) heteroaryl amine could impart acid-stability in the context of exceptional potency, we undertook a more systematic investigation of related modifications at C(4). Furans (**31** and **32**), regioisomeric thiophenes (**30**), and pyridines

Table 3
IGF-1R enzyme and phospho cellular IGF-1R IC_{50} results for **1**, **3**, and **9–16**



Compound	R	X	IGF-1R enzyme IC_{50} (nM)	Phospho GF-1R Cellular IC_{50} (nM)
1			2.0	117
2			7.9	30,000
9			79	2745
10			32	982
11			6.0	546
12			10	1770
13			13	773
14			250	2534
15			400	1695
16			25	654

Assay conditions used have been previously described and represent the average of ⩾2 independent measurements (see Ref. 4).

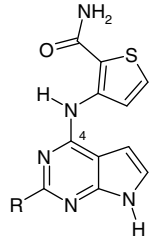


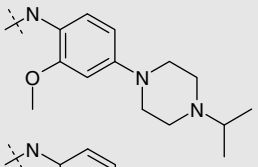
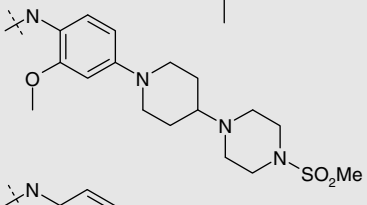
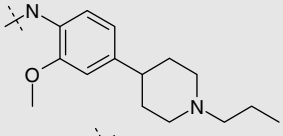
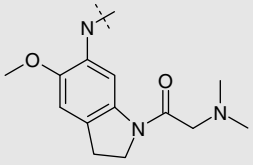
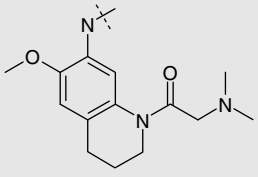
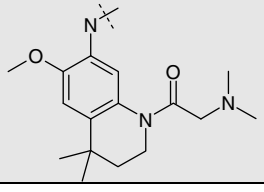
Scheme 2. General synthesis for heterocyclic C(4) anilines containing a carboxamide activating group.

(**35** and **36**) all proved more potent at both the enzyme and cellular level than benzamide **29** and could be synthesized in analogy to **23–28**. Additionally, these analogs showed a >100-fold improvement in acid stability over benzamide **29** with the exception of pyridyl analogs **35** and **36** (Table 5). Additionally, constrained lactam **37** retained both remarkable potency and stability. Finally, indolines **33** and **34** proved much more potent than initial indoline **12**, wherein the improvement in potency is likely attributable to a specific bidentate interaction between the *N*,*N*-dimethyl glycine moiety and Asp1056.[4]

In summary, a key developability issue was observed for potent IGF-1R inhibitor **1** wherein an acid-mediated cyclization of the pyrimidine moiety onto the pendant carboxamide led to facile hydrolysis in vitro and in vivo. Remarkable improvements in both inhibitor stability and potency were realized via substitution at C(4) with carboxamide-containing 5-membered heteroaryl amines (**23–28** and **30–32**), a constrained lactam (**37**), or indolines (**33** and **34**).[6] Further biological characterization and in vivo pharmacokinetics of this subclass of exceptionally potent and acid-stable inhibitors of IGF-1R will be reported in due course.

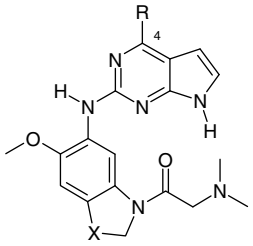
Table 4
IGF-1R enzyme and phospho cellular IGF-1R IC_{50} results for **23**–**28** (data represents ⩾2 independent measurements)

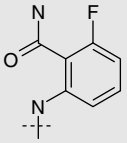
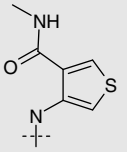
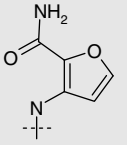
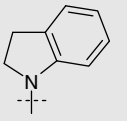
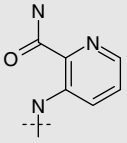
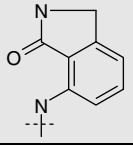


Compound	R	$t_{½}$ (h)	IGF-1R enzyme IC_{50} (nM)	Phosphol GF-1R Cellular IC_{50} (nM)
23		>1000	1.6	106
24		430	5.0	92
25		920	1.3	52
26		250	0.3	10
27		830	<0.2	6
28		720	0.2	<2

$t_{1/2}$ measured at 23 °C in 0.1 N HCl.

Table 5
IGF-1R enzyme and phospho cellular IGF-1R IC_{50} results for **29**–**37** (data represents ⩾2 independent measurements)



Compound	R	X	$t_{½}$ (h)	IGF-1R enzyme IC_{50} (nM)	Phosphol GF-1R Cellular IC_{50} (nM)
29		CH_2	2	1	54
30		CH_2	390	2	25
31		CH_2	>1000	0.8	28
32		CH_2CH_2	>1000	0.3	9
33		CH_2	>1000	0.8	160
34		CH_2CH_2	>1000	0.5	15
35		CH_2	15	0.7	38
36		CH_2CH_2	27	0.5	18
37		CH_2	>1000	0.8	16

$t_{1/2}$ measured at 23 °C in 0.1 N HCl.

References and notes

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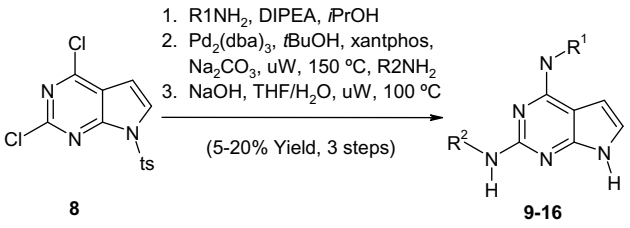
5. Analogs lacking a carboxamide could not be prepared as previously described; **9** was accessed by direct dehydration of the corresponding carboxmide with TFAA followed by neutralization with K_2CO_3/MeOH; **10**–**17** were accessed by initial base-mediated displacement with R^1NH_2 at C(4) followed by Buchwald–Hartwig amination using R^2NH_2 at C(6) and base-mediated detosylation.



6. The overall kinase selectivity for **1**, **4**–**7**, **9**–**16**, and **23**–**37** is relatively unchanged from the key molecules highlighted in Refs. 4,5. For example: (a) thiophene carboxamide **26** has a measured $IC_{50} \geqslant 1$ μM for 41 of 44 kinases tested, with IGF-1R, IR, and ALK giving IC_{50}s of 0.3, 0.4, and 0.4 nM respectively; (b) furan carboxamide **31** has a measured $IC_{50} \geqslant 1$ μM for 34 of 36 kinases tested, with IGF-1R and IR giving IC_{50}s of 0.8 and 0.8 nM, respectively (ALK not tested); (c) constrained lactam **37** has a measured IC_{50} of $\geqslant 1$ μM for 35 of 38 kinases tested with IGF-1R, IR, and ALK giving IC_{50}s of 0.8, 0.8, and 2.5 nM, respectively. For **26**, **31**, and **37** measured IC_{50}s for PI3K, GSK3, and AKT were all >10 μM.