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Imidazo[2,1-*b*]thiazoles: Multitargeted inhibitors of both the insulin-like growth factor receptor and members of the epidermal growth factor family of receptor tyrosine kinases

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ABSTRACT

The design and enzyme activities of a novel class of imidazo[2,1-*b*]thiazoles is presented.

Recent literature has suggested that simultaneous inhibition of the insulin-like growth factor receptor (IGF-IR) and members of the epidermal growth factor family (EGFR or ErbB2) with combinations of either small molecules or antibodies affords enhanced inhibition of cellular proliferation relative to the single agents.[1] In particular, the combination of IGF-IR and EGFR antibodies showed improved activity versus an A549 mouse tumor xenograft.[2] Combinations of gefitinib and NVP-ADW742 (selective EGFR and IGF-IR inhibitors, respectively) caused synergistic decreases in cellular proliferation across a diverse set of cancer cell lines.[3] The impressive combination data provides rationale for a small molecule discovery program targeting simultaneous inhibition of IGF-IR and EGFR/ErbB2. We have previously published on multitargeted pyrazolopyrimidines as inhibitors of IGF-IR, EGFR and ErbB2.[4] Herein, we disclose a novel scaffold that possesses the desired activities, similar to those highlighted above.

During a screen of Abbott's compound collection, we identified a scaffold, the imidazo[2,1-*b*]thiazoles, that were prepared as putative p38 kinase inhibitors.[5,6] Cross-screening of this class of compounds revealed that in addition to p38 inhibitory properties, appropriately substituted imidazothiazoles inhibited members of the ErbB-family of receptor tyrosine kinases (RTKs). According to the known SAR surrounding the ErbB-families of RTKs, the presence of a large hydrophobic head group is crucial for optimal activity versus both EGFR and ErbB2 (Fig. 1).[7] In contrast, a majority of

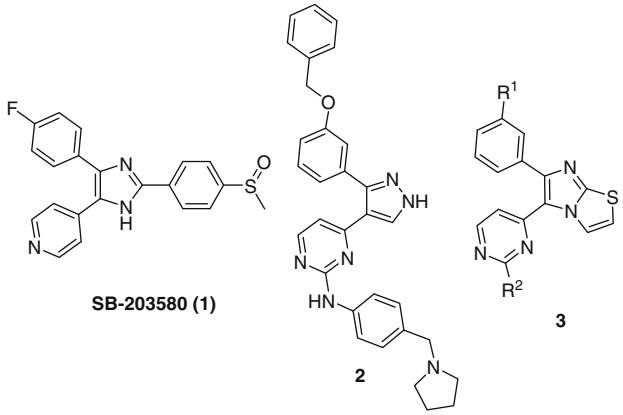

Figure 1. Structural comparison of SB-203580 (**1**), a literature IGF-IR inhibitor (**2**), and the desired imidazothiazole scaffold **3**.

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p38 scaffolds disclosed to date, like the prototypical p38 inhibitor, SB-203580 (**1**) require small substituents (like fluorine) for p38 inhibitory activity.[8] A survey of the literature revealed that proper modulation of the pendant functionality surrounding a reported p38 scaffold afforded compound **2,** which reportedly possessed enzyme activity versus IGF-IR.[9]

Pyrazole **2**, in combination with our screening data (vide supra), provided the impetus to execute a lead-optimization program geared toward targeting IGF-IR and members of the ErbB family of RTKs using a p38 scaffold as our lead. In doing so, we needed to develop a novel, flexible synthetic route toward highly substituted imidazothiazoles that would be required to afford a scaffold like **3**. From preliminary modeling studies, we envisioned that the R^1-substituent, head-piece, on **3** would access the hydrophobic pocket, and R^2-substituent, tail portion, would extend toward the solvent front of the kinase.

The initial set of analogs was prepared to probe the effect of putative hinge-binders on the kinase activity profile. The benzimidazole moiety in **4**, which has been postulated to serve as a hinge-binder for a PLK1 scaffold[10] was found to be inactive versus the targets of interest.[11] Replacement of the benzimidazole with a 4-pyridinyl group, as shown in **1**, provided analog **5**. This compound showed improved kinase inhibition versus the ErbB family of RTKs, however, was still inactive against IGF-IR. The use of substituted anilinopyrimidines provided improved potency against EGFR and ErbB2 activity, but still lacked IGF-IR activity (**6**–**7**). As expected from known SAR in the ErbB family, a simple nitro in the selectivity pocket abolished most ErbB2 activity (**8**). Finally, elaboration of the phenyl group by addition of a 4-morpholino-subsitutent provided **9**, the first analog to display enzyme inhibition versus all three targets of interest.

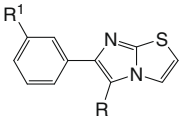
The initial SAR presented in Table 1 indicated that we needed a synthesis that was flexible enough to systematically vary both the amide head-group and the aniline tail-group. The synthesis of the imidazothiazole analogs proceeded from the condensation of 2-aminothiazole with the commercially available bromoketone **10** to afford **11** (Scheme 1). Freidel–Crafts acylation with acetic anhydride provided the methyl ketone **12**.[12] Subsequent treatment with DMF di-*tert*-butyl acetal provided enamine **13**, which was subjected to a three-step sequence entailing cyclization with guanidine, diazotization/hydrolysis, and chlorination with $POCl_3$ to provide chloropyrimidine **14**. Displacement of the chloride with substituted anilines, followed by nitro reduction and acylation afforded the fully elaborated imidazothiazole analogs (**6**–**9**, **16**–**30**).

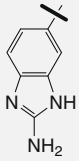
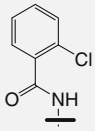
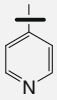
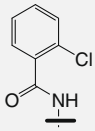
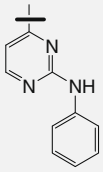
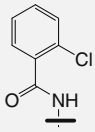
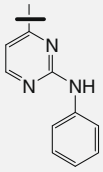
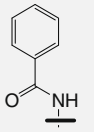
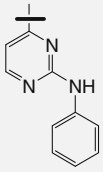
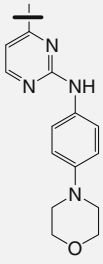
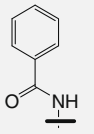
With the 2-anilinopyrimidine chosen as the preferred hinge binder, we examined a variety of groups in the amide position of our lead series (Table 2). Interestingly, 2,6-difluorobenzamide **16** was shown to be highly potent against ErbB2, and particularly against EGFR. However, benzamides generally inhibited IGF-IR fairly weakly. Cyclohexylmethyl (**17**) and cyclopentylmethyl (**18**) amides showed considerably weaker activity against EGFR and ErbB2. Phenyl urea (**19**) and phenyl carbamate (**20**) analogs exhibited reasonably balanced, albeit weaker, potency against all three enzymes. Phenylacetamide **21** demonstrated balanced and potent activity against all three enzymes, although it is less active against ErbB family kinases than **16**.

In order to assist in further SAR development, we obtained an EGFR X-ray co-crystal structure with analog **21**, which possessed a phenylacetamide head group. As shown in Figure 2, the amide head group lies deep in the protein in the selectivity/ hydrophobic pocket. The anilinopyrimidine forms two hydrogen bond interactions with the backbone of Met 769 in the hinge region of the protein. The morpholine tail of analog **21** extends to a solvent exposed region of the protein and close to Asp 776 and Glu 780 side chains. These two residues of EGFR correspond to Asp 786 and Glu 790 of ErbB2 and Ser 1059 and Ser 1063 of IGF-IR, respectively. While varied between EGFR/ErbB2 and IGF-IR, these side chains are all polar. Polar ligand–protein interactions in this region might modulate enzyme selectivity, but accurate prediction would not be possible because of the difficulty in quantitating polar interactions and desolvation effects in a solvent exposed environment. Thus IGF-IR potency could possibly be improved by addition of a polar tail to the 2,6-difluorobenzamides. Furthermore, the presence of the crystal structure confirmed that the most potent IGF-IR amide, the phenylacetamide, was tolerated by the ErbB-family of RTKs, confirming that this amide was worthy of further SAR exploration (vide infra).

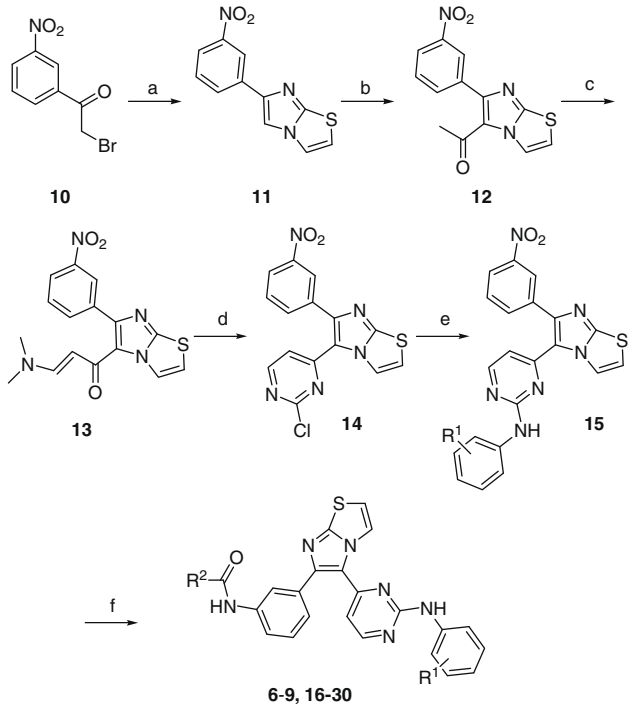
Exploration of a variety of tail groups, as illustrated by compounds **22**–**25** in Table 3 showed that a compound with a neutral group (**22**), exhibits low nM potency for EGFR and ErbB2, but only micromolar affinity for IGF-IR. Empirically, we found that cationic

Table 1
Kinase inhibitory activities of various hinge-binding motifs



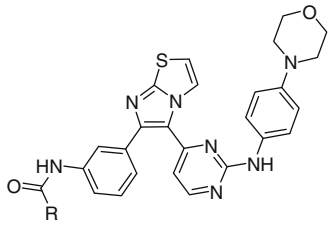
Analog	R=	R^1=	Enzyme (IC_{50}, nM)		
			IGF-IR	EGFR[a]	ErbB2
4			>12,500	>12,500	>12,500
5			>12,500	240	240
6			>12,500	83	35
7			>12,500	55	6.3
8		NO_2	>12,500	230	6000
9			1500	7.9	4.8

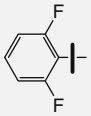
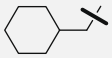
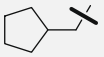
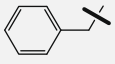
[a] EGFR kinase construct possessed the L858R activating mutation.



Scheme 1. Reagents and conditions: (a) 2-aminothiazole, NMP, 85 °C, 90%; (b) H_2SO_4, Ac_2O, 140 °C, 85%; (c) DMF-di-*tert*-butyl acetal, NMP, 90 °C, 89%; (d) 1-guanidine·HCl, K_2CO_3, NMP, 100 °C, 85%: 2-$NaNO_2$, AcOH, water, 60 °C, 90%; 3-$POCl_3$, 80 °C, 83%; (e) $ArNH_2$, HCl, 2-propanol, 35–90%; (f) 1-iron powder, NH_4Cl, EtOH, water, 90 °C, 50–88%; 2-RC(O)Cl, NMP, 45–99%

Table 2
Kinase inhibitory activities of 4-morpholino imidazothiazoles



Analog	R=	Enzyme (IC_{50}, nM)		
		IGF-IR	EGFR	ErbB2
9	Ph	1500	7.9	4.8
16		1300	1.9	86
17		1100	870	1400
18		580	660	1300
19	NHPh	590	100	170
20	OPh	450	190	170
21		130	63	370

tail groups, as shown in compounds **23**–**25**, improved the potency for all three enzymes by approximately an order of magnitude. Indeed, addition of an (*N*,*N*-dimethyl)ethylamine at the 4-position of the aniline (**23**) provided a >6-fold improvement in potency against IGF-IR compared to **16**, and a >15-fold potency improvement against ErbB2. An ethylpiperazine at the 4-position of the

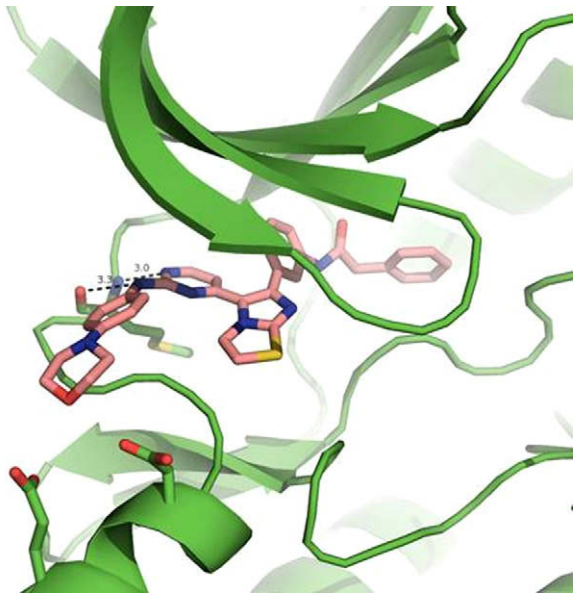
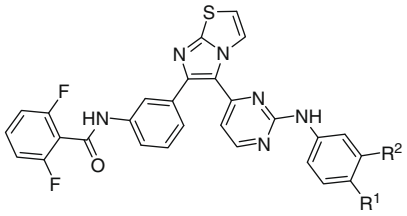
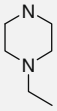


Figure 2. X-ray crystal structure of **21** in EGFR.[13]

Table 3
Kinase inhibitory activities of 2,6-difluorobenzamides

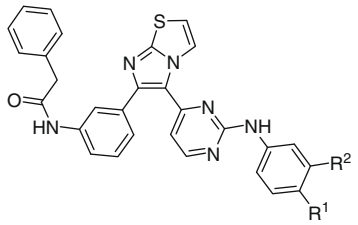


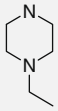
Analog	R^1=	R^2=	Enzyme (IC_{50}, nM)		
			IGF-IR	EGFR	ErbB2
22	H		3800	6.2	86
23		H	210	0.78	4.3
24		H	230	0.96	14
25			100	2.3	7.2

aniline (**24**) afforded a compound of similar potency to **23**. Incorporation of a fused ring secondary amine (**25**) further improved the IGF-IR potency twofold over **23**, while maintaining excellent EGFR and ErbB2 potency. Overall, the presence of an appropriately substituted aniline such as **25** provided an increase of 13-fold in potency against IGF-IR, and a 12-fold improvement against ErbB2 as compared to **16**, without a loss of enzyme activity versus EGFR. Unfortunately, this series of analogs did not afford the balanced enzyme profile that was desired.

We then returned to analogs possessing the phenylacetamide head group, which had previously been shown in compound **21** to provide a balanced, albeit weaker enzyme activity profile.

Table 4
Kinase inhibitory activities of phenylacetamides



Analog	R^1=	R^2=	Enzyme (IC_{50}, nM)		
			IGF-IR	EGFR	ErbB2
26	H		280	56	73
27			81	57	160
28		H	52	16	170
29	H		46	24	150
30			27	6.4	55

Optimization of the tail position, as in the benzamide series (Table 3), provided the analogs in Table 4. A fused ring tertiary amine (**27**) provided a small improvement in IGF-IR and ErbB2 potency compared to **21**, without loss of EGFR activity. An ethylpiperazine in the 4-position of the aniline (**28**) provided a further boost to IGF-IR and EGFR potency. The secondary amine **30** provided a uniform increase in potency compared to **21** of at least fivefold, also providing a compound with balanced activity of <100 nM against all three enzymes.

Selected compounds were further characterized by their ability to prevent target phosphorylation in cellular systems.[11] As shown in Table 5, inhibition of intracellular phosphorylation correlated well with kinase inhibitory activity. Several compounds were active in the cellular assay at <400 nM across all three kinases. Surprisingly, although the phenylacetamides (**26**,[14] **28, 30**) appear to have a more balanced activity profile in the enzyme assay, the 2,6-difluorobenzamides (**23**, **24**) were as balanced in the cellular assay. Furthermore, compound **28** approaches the cellular activity of OSI-906 against IGF-1R, and compounds **23** and **24** are as potent as lapatinib and erlotinib against EGFR and ErbB2. It should also be noted that while the EGFR kinase assays were performed using the L858R mutation, the A431 cells used in the cellular assay possess wild-type EGFR.

In conclusion, we have disclosed that suitably functionalized imidazothiazole scaffolds provide potent and balanced enzyme and cellular activity against IGF-IR, EGFR, and ErbB2. The selectivity profile has been shown to be highly dependent upon the amide head group. In addition, as suggested by a crystal structure of compound **21** bound to EGFR, the incorporation of an appropriately functionalized amine tail provided the desired activity profile. Further improvements in potency and selectivity in this and related series will be reported in due course.

Table 5
Inhibition of cellular phosphorylation

Analog	IC_{50}[a] (nM)		
	pIGF-1R	p-EGFR	p-ErbB2
23	330	220	27
24	200	50	69
26	140	610	680
28	41	350	310
30	180	1000	120
Erlotinib	>10,000	34	5200
Lapatinib	>10,000	52	100
OSI-906	18	>10,000	>10,000

[a] pIGF-1R and pEGFR were performed in A431 epidermoid carcinoma cells. pErbB2 was performed in N87 gastric cancer cells.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.015.

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11. The kinase activities of disclosed compounds were assayed by a homogeneous time-resolved fluorescence assay as previously described. The inhibition of intracellular phosphorylation by selected compounds was assayed using ELISA as previously described: Hubbard, R. D.; Bamaung, N. Y.; Palazzo, F.; Zhang, Q.; Kovar, P.; Osterling, D. J.; Hu, X.; Wilsbacher, J. L.; Johnson, E. F.; Bouska, J.; Wand, J.; Bell, R. L.; Davidsen, S. K.; Sheppard, G. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5406.
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13. The refined crystallographic coordinates have been deposited in the Protein Data Bank (www.rcsb.org) with entry code 3LZB.
14. Compound **26** has been tested against a panel of 56 kinases for selectivity. Data can be found in Supplementary data.