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Discovery of novel isothiazole inhibitors of the TrkA kinase: Structure–activity relationship, computer modeling, optimization, and identification of highly potent antagonists

Blaise Lippa,* Joel Morris, Matthew Corbett, Tricia A. Kwan, Mark C. Noe, Sheri L. Snow, Thomas G. Gant, Melchiorra Mangiaracina, Heather A. Coffey, Barbara Foster, Elisabeth A. Knauth and Matthew D. Wessel

Pfizer Inc., PGRD Groton, MS-8220-2203 Eastern Point Rd., Groton, CT 06340, USA

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Abstract—The design, synthesis, and biological evaluation of potent inhibitors of the TrkA kinase is presented. A homology model is created to aid in the enhancement of potency and selectivity of isothiazole inhibitors found during a high-throughput screen. Three different syntheses are utilized to make diverse analogs within this series. Aminoheterocycles are found to be good urea surrogates, whereas bicyclic substituents on the C3 thio group were found to be extremely potent TrkA inhibitors in kinase and cell assays.
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Prostate and pancreatic cancers are two of the most common forms of cancers in the US and will kill an estimated 62,000 Americans in 2005.[1] In the case of pancreatic cancer, little progress has been made over the past 50 years in the treatment of this particularly painful disease, which has a 5-year survival rate of 4.6% from first diagnosis.[1] Prostate cancer is the most common non-cutaneous malignancy in men in the US, and when tumors become resistant to anti-hormonal therapy, other non-hormone related treatment options are severely limited.[2] Clearly, new therapies are urgently needed to treat these diseases.

TrkA is a part of a family of receptor tyrosine kinases that also includes TrkB and TrkC. These kinases are receptors for the neurotrophin family of ligands, which includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins 3–5.[3] Members of this family of ligands and receptors are found to be up-regulated in a variety of human cancers, particularly prostate[4] and pancreatic cancers,[5] and are thought to play a key role in the progression of those diseases. In the case of pancreatic cancer, increased expression of TrkA also correlates with an increased level of pain. Staurosporine Trk inhibitors from Cephalon have showed excellent preclinical anti-tumor efficacy[6,7] and have entered human clinical trials.[8] However, few Trk inhibitors are known and very few show kinase selectivity.[9–11]

The therapeutic implications of an effective Trk inhibitor may well go beyond cancer therapy. The link of the Trks and their ligands with CNS related processes has implicated this signaling cascade with a range of other diseases, most notably pain.[12] Because of the therapeutic promise associated with inhibiting TrkA, and the relative lack of potent and selective inhibitors, a high-throughput screen was initiated which revealed that several isothiazoles were effective in inhibiting the TrkA kinase. Herein is reported the synthesis and analysis of several series of potent and selective TrkA kinase inhibitors.

To better understand how the original lead isothiazole inhibitor (**1**) would bind to TrkA, a homology model of the kinase domain was constructed from insulin receptor (IR-pdb code: 1IRK). The homology model was built by first aligning the primary sequences of

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* Corresponding author. Tel.: +1 860 715 4660; fax: +1 860 686 6258; e-mail: blaise.lippa@pfizer.com

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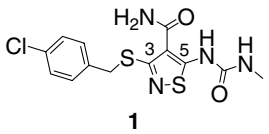

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Figure 1. Lead isothiazole inhibitor of the TrkA kinase, found through a high-throughput screen.

TrkA with IR, and then constructing the 3D structure of TrkA based on the 3D structure of IR, using the homology model interface within the MOE software suite.[13] Figure 2 depicts the active site of TrkA with **1** in a putative binding conformation. The unsubstituted carboxamide of **1** makes two key hydrogen bonds with the same residues of the protein backbone where ATP would normally interact. This proposed binding mode is supported by previous SAR studies that have shown that replacements for this carboxamide such as CN or tetrazole, or even a methylcarboxamide are universally not tolerated. This carboxamide also appears to rigidify the inhibitor through an intramolecular hydrogen bond with the internal nitrogen of the urea. The urea appears to be pointed toward a solvent exposed region, whereas the *p*-chlorobenzyl substituent is directed into a portion of the protein where the sugar of ATP normally binds. These orientations are in accord with SAR observations at C5 that lipophilic urea substituents have decreased potency.

Notably, this docked structure suggested that there was an un-utilized lipophilic pocket in the vicinity of the benzylic carbon. Thus, analogs were designed to optimally fill this space. In addition, the conformation of the urea suggested that it could be replaced by other functional groups, such as aminoheterocycles.

The initial synthesis of thiosubstituted isothiazole ureas is shown in Scheme 1. Compound **2** is made through the addition of *p*-methoxybenzylthiol with malonitrile. This intermediate is then reacted with PhOCONCS followed by ring closure with iodine to result in the isothiazole **3**. Thiol deprotection is accomplished with mercury acetate, TFA, and hydrogen sulfide. The cyanide is then hydrolyzed with sulfuric acid to provide the key intermediate **4**. Functionalization of the thiol is accomplished under mildly basic conditions with benzyl halides, and urea formation occurs through the addition of amines to result in final analogs **5a**–**h** (Table 1).

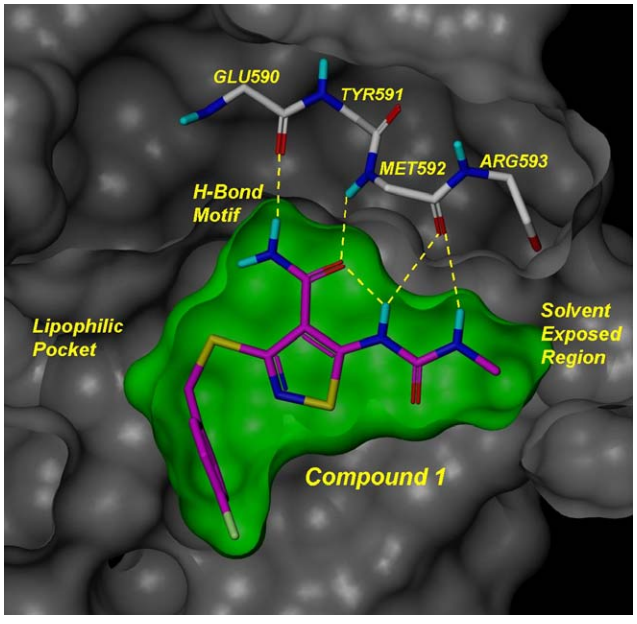
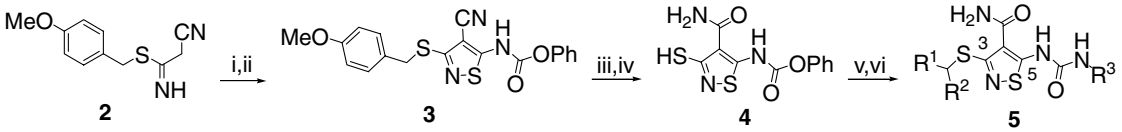


Figure 2. Compound **1** docked into a homology model of TrkA.

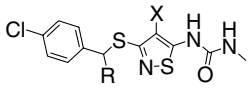
The original lead **1** was shown to be a potent inhibitor in the TrkA kinase assay[14] and also relatively potent in the TrkA cell assay.[15] Unfortunately, it also had some kinase selectivity issues, particularly the VEGFR-2 kinase[16] where it was only 4× selective. Substitution on the C3 benzylic carbon produced remarkable effects in potency and particularly VEGFR-2 kinase selectivity. Substitution with methyl or ethyl at this position retained kinase potency and enhanced cellular TrkA potency. Substitution with *i*-Pr was tolerated, whereas *n*-Pr, CF_3, and Ph substitution resulted in a loss of potency. Importantly, all substituents at this position showed a large increase in VEGFR-2 kinase selectivity from 4× (R = H, **1**) to as high as 111× when R = Et (**5b**). For synthetic ease, all compounds were originally synthesized as racemates. However, it was possible to utilize chiral chromatography to separate enantiomers. Thus, **5b** was separated to provide the *R* enantiomer **5c** and the *S* enantiomer **5d**. The *R* enantiomer (**5c**) was determined to be >10× more potent than the *S* enantiomer, and VEGFR-2 kinase selectivity was increased to 1300×. Presumably, these benzylic substituents are filling the lipophilic pocket on TrkA, as predicted (Fig. 1). The reason for increased selectivity over VEGFR-2 appears to be caused by a difference in this lipophilic pocket between VEGFR-2 and TrkA. It is not entirely clear, but the pocket in VEGFR-2 is composed mainly of aliphatic residues (Val, Ile, and Leu). In the TrkA pocket, at least three Phe residues exist in place of these aliphatic VEGFR-2 residues. This is undoubtedly causing a change in the size, shape, and nature of this pocket between the two proteins, thus potentially leading to the selectivity trends observed. As previously mentioned, the C4 cyanide analog **6** showed essentially no TrkA potency, presumably due to its lack of the key carboxamide hydrogen bond donor–acceptor motif.

Based on the docked structure of **1**, aminoheterocyclic replacements for the urea were then targeted to further elucidate the binding mode of inhibitors and to improve ADME properties. Scheme 2 depicts a first generation synthesis that allowed late-stage incorporation of diverse C5 aminoheterocycles, while conserving the most potent substituents at the other isothiazole positions. Compound **7**[17] was first hydrolyzed to the methyl ester and both sulfurs were oxidized to sulfones. It has been shown previously that aromatic methylsulfones are excellent leaving groups when participating in nucleophilic aromatic substitutions, and that the C5 sulfone is more reactive than the C3 sulfone.[17] Thus, displacement of the C5 methylsulfone of **8** with ammonia in



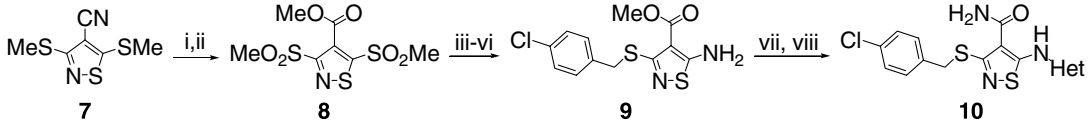
Scheme 1. The synthesis of isothiazole analogs. Reagents: (i) PhOCONCS, 70%; (ii) I_2, pyridine, 91%; (iii) $Hg(OAc)_2$, H_2S, TFA, 95%; (iv) H_2SO_4, 85%; (v) R^1R^2CHX, $(i\text{-Pr})_2NEt$; (vi) R^3NH_2.

Table 1. TrkA kinase and cell inhibiton, and VEGFR-2 kinase selectivity for isothiazoles **1**, **5a**–**h**, and **6**



Compound	X	R[a]	TrkA kinase inhibition IC_{50}[b] (nM)	TrkA cell inhibition IC_{50}[b] (nM)	VEGFR-2 kinase selectivity[b]
1	$CONH_2$	H	7	300	4×
5a	$CONH_2$	Me	3	96	57×
5b	$CONH_2$	Et	9	70	111×
5c	$CONH_2$	Et[c]	4	46	1300×
5d	$CONH_2$	Et[d]	52	978	10×
5e	$CONH_2$	*i*-Pr	16	290	188×
5f	$CONH_2$	*n*-Pr	35	600	71×
5g	$CONH_2$	CF_3	110	2870	NT
5h	$CONH_2$	Ph	2600	NT	NT
6	CN	H	>5000	NT	NT

(NT, not tested).
[a] Compounds were tested as racemic mixtures, unless otherwise noted.
[b] Values are means of at least two experiments, assay error is <2×.
[c] *R* enantiomer.
[d] *S* enantiomer.

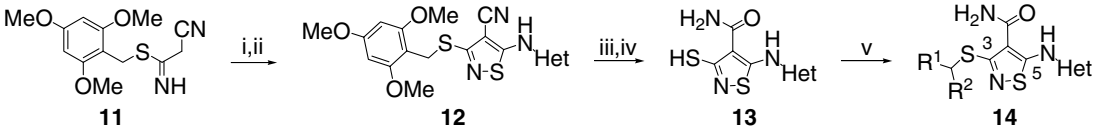


Scheme 2. The synthesis of aminoheterocycle-substituted isothiazoles. Reagents: (i) H_2SO_4, MeOH, 93%; (ii) oxone, 94%; (iii) NH_3, THF, 75%; (iv) NaH, Boc_2O, 82%; (v) *p*-ClBnSH, *n*-BuLi, 60%; (vi) TFA, CH_2Cl_2, 86%; (vii) HetArBr, $Pd_2(dba)_3$, *rac*-BINAP, Cs_2CO_3, Tol; (viii) Me_3Al, NH_4Cl, Tol.

THF, Boc protection, and then displacement of the C3 sulfone with *p*-chlorobenzylthiol results in compound **9** in good yield after Boc deprotection. Notably, the ammonia displacement must be done in THF; early attempts with methanol resulted in bis addition of ammonia to two isothiazoles, both at the C5 position. Standard Pd-mediated aminations then are successful in creating the amino-heterocycle bond,[18] and the analog is completed through a Weinreb amidation to give **10**.

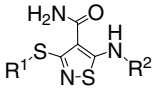
While the original route to the aminoheterocycle-substituted isothiazoles allowed late-stage examination of the aminoheterocycle portion of analogs, it did not allow late-stage modulation of the C3 thio substituent. Thus, a second synthetic method was created to allow facile variation of the C3 thio substituent with a fixed aminoheterocycle at C5. In designing such a scheme, there was a desire to avoid the harsh C3 sulfur deprotection conditions used previously in Scheme 1. Thus, a 2,4,6-trimethoxybenzyl-protecting group was utilized in place of the *p*-methoxybenzyl group (Scheme 3). Compound **11** was reacted with a heteroaryl isothiocyanate followed by ring closure with iodine to yield compound **12**. The cyanide was then hydrolyzed with sulfuric acid, and the crude product was subjected to relatively mild deprotection conditions: 10% TFA in dichloromethane in the presence of triethylsilane. This procedure cleanly deprotected the C3 thiol in 73% yield (two steps). Notably, it was found that the triethylsilane is absolutely required to scavenge the benzyl cation as it is formed and to prevent the reversibility of the deprotection reaction. The thiol can then be capped with a variety of electrophiles under standard conditions to examine the effects of the C3 substituent.

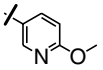
Table 2 shows TrkA kinase and cell potency for a range of C5 aminoheterocycles. Notably, a diverse set of aminoheterocycles are shown to be adequate urea surrogates. Surprisingly, all aminoheterocycles revealed similar TrkA kinase and cell potency. Variation of the C3 sulfur substituent in this series showed that the replace-



Scheme 3. Alternate synthesis of aminoheterocycle-substituted isothiazoles. Reagents: (i) (Het)NCS, EtOAc, 60%; (ii) I_2, pyridine, 81%; (iii) H_2SO_4; (iv) Et_3SiH, TFA, CH_2Cl_2, 73%—two steps; (v) R^1R^2CHX, $(i\text{-Pr})_2NEt$, DMF.

Table 2. TrkA kinase and cell inhibition for aminoheterocycle isothiazole analogs **10a–g** and **14a–c**



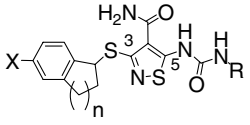
Compound	R^1	R^2	TrkA kinase inhibition IC_{50}[a] (nM)	TrkA cell inhibition IC_{50}[a] (nM)
10a	*p*-Chlorobenzyl	H	>5000	NT
10b	*p*-Chlorobenzyl	2-pyr	33	756
10c	*p*-Chlorobenzyl	3-pyr	19	581
14a	CH(Me)(*p*-chlorobenzyl)	3-pyr	29	NT
14b	CH_2(cyclohexyl)	3-pyr	610	>1000
10d	*p*-Chlorobenzyl	4-pyr	45	344
14c	*o*-Chlorobenzyl	4-pyr	28	NT
10e	*p*-Chlorobenzyl	4-pyrimidine	47	NT
10f	*p*-Chlorobenzyl		179	>1000
10g	*p*-Chlorobenzyl		84	NT

(NT, not tested).
[a] Values are means of at least two experiments, assay error is <2×.

ment of the phenyl ring with a saturated cyclohexyl group was not tolerated (**14b**), whereas an *o*-chlorobenzyl group was well tolerated (**14c**).

While the aminoheterocyclic urea replacements did demonstrate that the urea could be replaced with other functionality, TrkA potency was not increased, and ADME properties were not significantly improved. To further enhance potency, attention turned back to the C3 substituent. Based on the knowledge that substitution of the benzylic position was beneficial (Table 1), this structural motif was retained, while cyclizing the benzylic substituent back onto the phenyl ring to create a bicyclic moiety, thus further rigidifying the structure. Utilizing the synthetic pathway of Scheme 1, several such analogs were synthesized in the C5 urea series (Table 3). Compounds **5i** and **5j** largely retained potency when compared to the acyclic ethyl derivative **5b**. In these cases, the potency of the methylated and unsubstituted urea were similar. Enlarging the ring to a 6-membered size resulted in a ~10× decrease in potency (**5k** and **5l**). However, further ring enlargement to a 7-membered ring provided the most potent TrkA inhibitors synthesized to date. An approximately 10× improvement in potency from the 5-membered ring is realized and a <1 nM kinase potency is achieved along with 17 nM cell potency (**5m**). Separation of these enantiomers again showed that the majority of the potency resides in the *R* enantiomer (**5n**), and a further increase in potency, now to 7 nM in the cell, is achieved. It should also be noted that, although not tested regularly, compounds in all series tended to inhibit TrkB with similar potency. TrkC selectivity was not determined.

Table 3. TrkA kinase and cell inhibiton for isothiazoles with sulfur linked C3 bicycles **5i-o**



Compound[a]	X	n	R	TrkA kinase inhibition IC_{50}[b] (nM)	TrkA cell inhibition IC_{50}[b] (nM)
5i	Cl	1	H	10	110
5j	Cl	1	Me	8	118
5k	H	2	H	105	1170
5l	H	2	Me	111	1020
5m	H	3	H	<1	17
5n[c]	H	3	H	<1	7
5o[d]	H	3	H	91	>1000

[a] Compounds were tested as racemic mixtures, unless otherwise noted.
[b] Values are means of at least two experiments, assay error is <2×.
[c] *R* enantiomer.
[d] *S* enantiomer.

In conclusion, a homology model was utilized to design highly potent and selective inhibitors of the TrkA kinase. These compounds were synthesized through three novel synthetic routes, which allowed access to diverse functionality. Aminoheterocycles were shown to be good urea surrogates for inhibiting TrkA, whereas C3-thiosubstituted bicycles were shown to be extremely potent TrkA inhibitors, on par or better than all other known TrkA inhibitors. These compounds should offer the scientific community excellent tools for further understanding the TrkA signaling cascade, and the implications for inhibiting this pathway. Further in vivo studies are needed to determine if these inhibitors will have desirable anti-cancer therapeutic benefits.

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