Bioorganic & Medicinal Chemistry Letters 20 (2010) 1669–1673

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Replacement of pyrazol-3-yl amine hinge binder with thiazol-2-yl amine: Discovery of potent and selective JAK2 inhibitors

Stephanos Ioannidis [a,*], Michelle L. Lamb [a], Lynsie Almeida [a], Huiping Guan [a], Bo Peng [a], Geraldine Bebernitz [b], Kirsten Bell [b], Marat Alimzhanov [b], Michael Zinda [b]

[a] Department of Cancer Chemistry, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA
[b] Department of Cancer Bioscience, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA

ARTICLE INFO

Article history:
Received 10 December 2009
Revised 7 January 2010
Accepted 11 January 2010
Available online 25 January 2010

Keywords:
Pyrazol-3-yl amine
Thiazol-2-yl amine
JAK2 inhibitors
AZ960

ABSTRACT

Thiazol-2-yl amine was identified as an isosteric replacement for pyrazol-3-yl amine during our efforts to identify potent and selective JAK2 inhibitors. The rationale, synthesis and biological evaluation of several analogs is reported, along with the in vivo evaluation of the lead compounds.

As part of our efforts to identify potent and selective JAK2 (Janus Kinase 2) inhibitors, we have recently described pyrazol-3-yl amino nicotinonitrile (**AZ960**, Fig. 1) and pyrazol-3-yl amino pyrazines as ATP-competitive compounds.[1,2] Based on past experience with kinase inhibitors,[3] we anticipated that for both scaffolds, the pyrazol-3-yl amine group occupies the ATP binding site and interacts via hydrogen bonds with the JAK2 protein backbone (hinge). More specifically, three hydrogen bond interactions in a cis-donor/acceptor/donor motif were expected with the pyrazol-3-yl amine. Based upon this binding mode, any substituents at the C_5 of the pyrazole ring would be stacked against the selectivity pocket gatekeeper, which for JAK2 is a methionine residue. Earlier work by Pierce et al.[4] with quinazoline-containing kinase inhibitors suggested for this binding motif that a further intra-molecular hydrogen bond between the C_4–H of the pyrazole and the adjacent nitrogen of the pyridine/pyrazine might be crucial for activity (see Fig. 1). This interaction would lock the two rings into a co-planar conformation and permit efficient interaction of the pyrazole with the hinge.

Interestingly, these authors found that a thiazole could replace the pyrazole and retain potency for their quinazoline analogs. This was explained by the aromatic C–H adjacent to a heteroatom participating in a hydrogen bond as donor[4,5] and a favorable electrostatic interaction between the sulfur and the nitrogen of the adjacent ring.[4] With this background, we considered that bioisosteric replacement of the pyrazol-3-yl amine group with thiazol-2-yl amine group should result in potent JAK2 inhibitors with our pyrazine scaffold.[2] The thiazol-2-yl amine moiety is present in several reported kinase inhibitors such as dasatinib.[6]The hypothesis was the cis-donor motif would be retained by the C_2–NH and C_4–H of the thiazole with the predicted increase in the basic character of thiazole nitrogen enhancing the acceptor potential with the backbone, and balancing the weaker acidity of the C–H bond.

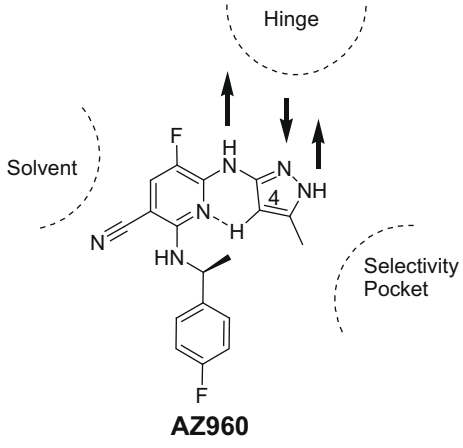


Figure 1. Intramolecular hydrogen bond between C-4 and nitrogen of the pyridine locks the two rings in co-planar conformation in AZ960.

* Corresponding author. Tel.: +1 781 839 4556; fax: +1 781 839 4630.
E-mail address: Stephanos.Ioannidis@astrazeneca.com (S. Ioannidis).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.091

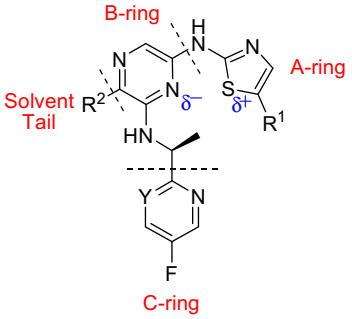


Figure 2. Proposed electrostatic interaction between the N (δ−) of the pyrazine and the sulfur (δ+) of the thiazole.

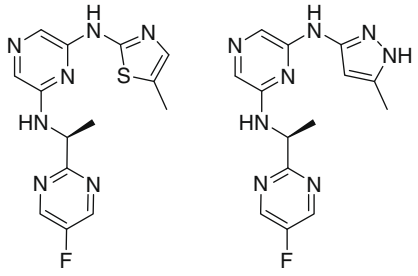
Likewise, the co-planarity between the thiazole and the B-ring (Fig. 2) would be sustained via a favorable electrostatic interaction between the nitrogen (δ−) of the B-ring and the sulfur of the thiazole (δ+) as was seen with the pyrazol-3-yl quinazoline inhibitors.

We envisioned that prompt confirmation of whether the thiazole replacement is tolerated within the pyrazine series could be determined by the synthesis of compound **3** (Scheme 1).

Compound **3** was readily accessible via nucleophilic aromatic substitution of 2,6-dichloropyrazine with the chiral amine **1** under microwave conditions. The thiazole moiety was installed by a standard palladium-catalyzed amination reaction. We were gratified to find that compound **3** showed excellent JAK2 inhibition, comparable to compound **4**.

As shown in Table 1, both molecules were potent in the JAK2 enzyme assay at K_m ATP,[1] indicating that the thiazol-2-yl group binds sufficiently to the hinge. The thiazole showed decreased potency in JAK2 inhibitory activity at high concentrations of ATP in comparison with the corresponding pyrazole analog. However, it was not possible to estimate the exact drop off in potency since JAK2 inhibition at K_m was at the detection limit of the assay. It would be expected that for a tightly-bound, ATP-competitive inhibitor, the drop off in JAK2 potency between K_m ATP and high ATP (5 mM) concentrations would be small. The use of a high concentration of ATP in JAK2 enzyme screening was anticipated to mimic the cellular environment and allow more accurate differentiation between the compounds tested. Thus, we envisaged that reduced inhibitory activity in the high ATP JAK2 enzyme assay might translate into weaker cellular activity. Hence we were not surprised when testing compound **3** in a TEL-JAK2 proliferation assay revealed only moderate activity (GI_{50} ~0.37 μM). In vitro screening in rat microsomes showed the compound to have moderate intrinsic clearance (CL_{int} = 52 μL/min/mg). It was known from previous studies that the substituents at R^2 (Fig. 2) could effectively improve cellular potency as well as metabolic stability. In

Table 1
Comparison of thiazol-2-yl pyrazine and pyrazol-3-yl pyrazine **3** and **4**



3 **4**

Compd	JAK2 IC_{50}[a] (μM)	JAK2 (5 mM ATP) IC_{50}, μM	Solubility[b] (μM)
3	0.004	0.049	>1000
4	<0.003	0.003	>1000

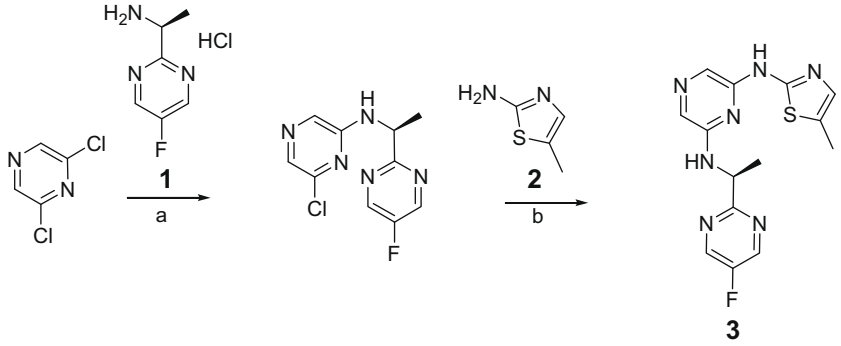
[a] At K_m ATP concentration.
[b] Obtained from solid after drying its DMSO solution.

an attempt to improve these properties in the thiazole series, we considered the installation of several groups (such as morpholine) at the solvent tail position R^2. Despite our efforts, a viable synthetic route allowing attachment of a solvent tail to the pyrazine scaffold was not identified; therefore, we turned our attention to thiazol-2-yl pyrimidines in order to test our hypothesis.

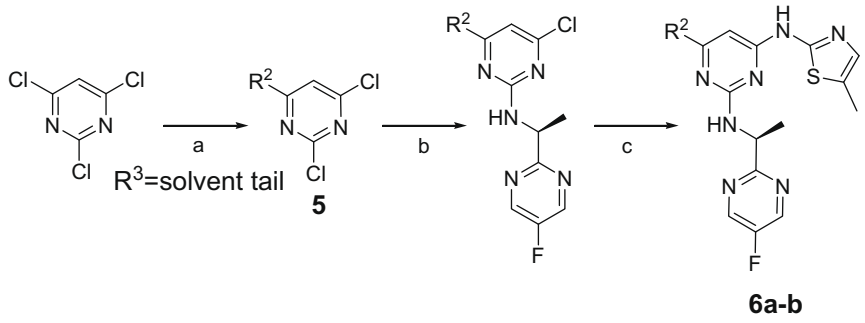
We anticipated that introduction of the solvent tail first, followed by the addition of the C-ring and subsequent palladium-catalyzed amination would furnish the desired thiazol-2-ylamines as shown in Scheme 2. Reaction of amine **1** with intermediate **5** resulted in the formation of two regio-isomers with the chiral amine attached either at the 2- or 4-position of the pyrimidine (only the desired regio-isomer is depicted in Scheme 2). Separation of the regio-isomers was readily accomplished by column chromatography. Table 2 illustrates the JAK2 enzymatic and cellular (TEL-JAK2) potencies together with a measure of JAK3 selectivity, as determined in a TEL-JAK3 proliferation assay in Ba/F3 cells.[1] This measurement was triggered because of the potential for immunosuppression due to attenuated JAK3 activity.

Both **6a** and **6b** showed excellent JAK2 activity at high ATP concentrations, implying tight binding of these analogs with the JAK2 protein. In addition, the nature of the substituent at the solvent tail position appeared important in determining the level of selectivity versus JAK3. The morpholine analog **6b** exhibited excellent potency against JAK2 along with exquisite selectivity versus JAK3. In contrast, the JAK2 potency of **6a** did not appear to translate to the cellular level.

We extended our efforts by replacing the (*S*)-5-fluoropyrimidin-2-yl-ethanamine C-ring with (*S*)-5-fluoropyridin-2-yl-ethanamine.

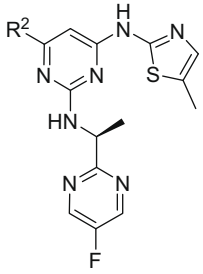


Scheme 1. Reagents and conditions: (a) NMP, Hünig's base, 180 °C, microwave; (b) Cs_2CO_3, $Pd_2(dba)_3$, Xantphos®, dioxane, 100 °C.



Scheme 2. Reagents and conditions: (a) for **6a**: NaOMe, MeOH, 0–25 °C; for **6b**: morpholine, EtOH, −20 °C to 0 °C; (b) (*S*)-5-fluoropyrimidin-2-yl-ethanamine hydrochloride 1, Hünig's base, *n*-BuOH, 120 °C; (c) 5-methyl-1,3-thiazol-2-amine, Cs_2CO_3, $Pd_2(dba)_3$, BINAP, dioxane, 95 °C.

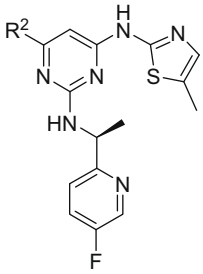
Table 2
Enzymatic and cellular evaluation of (*S*)-N^2-(1-(5-fluoropyrimidin-2-yl)ethyl)-N^4-(5-methylthiazol-2-yl)pyrimidines



Compd	R^3	JAK2[a] IC_{50}, μM	TEL-JAK2 GI_{50} (μM)	TEL-JAK3 GI_{50} (μM)	JAK3 fold selectivity[b]
6a	OMe	<0.003	0.042	1.5	35
6b	Morpholine	0.003	0.005	1.1	237

[a] At 5 mM ATP concentration.
[b] Calculated from TEL-JAK3 to TEL-JAK2 ratio.

Table 3
Enzymatic and cellular evaluation of (*S*)-N^2-(1-(5-fluoropyridin-2-yl)ethyl)-N^4-(5-methylthiazol-2-yl)pyrimidines



Compd	R^3	JAK2[a] IC_{50} (μM)	TEL-JAK2 GI_{50} (μM)	TEL-JAK3 GI_{50} (μM)	JAK3 fold selectivity[b]
7a	1-Methyl piperazine	<0.003	0.005	0.62	129
7b	Morpholine	0.005	0.005	1.2	243

[a] At 5 mM ATP concentration.
[b] Calculated from TEL-JAK3 to TEL-JAK2 ratio.

Analogs **7a–b** were prepared according to the procedures described in Scheme 2 for the corresponding (*S*)-5-fluoropyrimidin-2-yl-ethanamine derivatives.

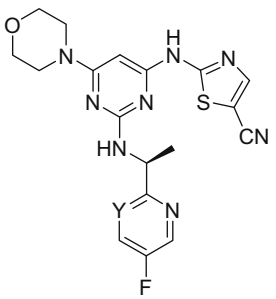
Interestingly, while the morpholine analog **7b** showed excellent selectivity vs JAK3, the 1-methylpiperazine, although very potent, seemed to be less selective (Table 3).

The methyl group of the thiazole is presumed to occupy the selectivity pocket. It was speculated that only small groups could be accommodated in the selectivity pocket due to steric interactions with the Met gatekeeper in JAK2. Hence, we investigated replacement of 5-methylthiazol-2-amine with 2-aminothiazole-5-carbonitrile.[7] The morpholine solvent tail was retained due to the excellent potency (**6b** and **7b**) and selectivity (see **6b** vs **6a** and **7b** vs **7a**) against JAK3 demonstrated by the analogs described above. The effect of the two different C-rings was also tested (Table 4).

To our surprise, incorporation of the cyano group appeared to result in both a reduction in JAK2 activity, and a loss of selectivity against JAK3.

The thiazol-2-yl pyrimidines were screened in an in vitro metabolic stability assay in rat microsomes and their potential to inhibit the hERG channel was assessed. In addition, the pharmacokinetic (PK) profile of the most potent and selective JAK2 inhibitors (**6b**, **7a** and **7b**) was determined following iv dosing in rats (Table 5). All compounds containing the 5-methylthiazol-2-amine hinge binder displayed high intrinsic clearance, while the 2-aminothiazole-5-carbonitrile analogs (**8a** and **8b**) appeared to be more stable. This is possibly because the methyl group is prone to oxidation by CYP450 enzymes while such biotransformation is not possible for the cyano group.[8] Likewise, comparing the rat microsomal stability between **3** and **4** (Table 5) suggests the metabolic instability of 5-methylthiazol-2-amine.

Table 4
The effect of 2-aminothiazole-5-carbonitrile hinge binder



Compd	Y	JAK2[a] IC_{50} (μM)	TEL-JAK2 GI_{50} (μM)	TEL-JAK3 GI_{50} (μM)	JAK3 fold selectivity[b]
8a	CH	<0.003	0.036	0.17	5
8b	N	0.079	0.053	0.52	10

[a] At 5 mM ATP concentration.
[b] Calculated from TEL-JAK3 to TEL-JAK2 ratio.

Table 5
Rat microsomal stability,[a] PK parameters, [b] hERG and lipophilicity evaluation of thiazol-2-yl pyrimidines

Compd	Rat CLint (μL/min/mg)	Rat CL (ml/min/Kg)	Rat $T_{1/2}$ (h)	Vdss (L/Kg)	hERG IC_{50} (μM)	*c* log *P*
3	52	NT	NT	NT	NT	1.75
4	<4	14	1.4	1.1	>32	1.25
6a	>100	NT	NT	NT	>32	2.68
6b	84	32	1.3	2.3	>32	1.94
7a	220	80	1.3	8.5	13	3.39
7b	>120	38	1.2	2.8	12	2.95
8a	31	NT	NT	NT	>32	1.93
8b	41	NT	NT	NT	NT	0.94

[a] The time-dependent disappearance of compound (2 μM initial concentration) incubated with microsomes is measured by LC–MS/MS.
[b] Han Wistar rat male; 3 mg/kg iv.

Results from the in vivo PK screening revealed a lack of correlation between the in vitro and in vivo determinations for the thiazole analogs. Compounds **6b** and **7b** showed moderate plasma stability while compound **7a** was highly cleared from the plasma. The only difference between **7a** and **7b** is the 1-methylpiperazine versus the morpholino solvent tail, suggesting that the 1-methylpiperazine may be more susceptible to metabolism.

From the results shown in Table 5, it also appeared to us that incorporation of the (*S*)-5-fluoropyrimidin-2-yl-ethanamine C-ring resulted in negligible inhibition of the hERG potassium channel. In contrast, the more basic pyridine C-ring (**7a**–**7b**) along with increased lipophilicity as indicated by calculated log P (*c* log P) led to compounds having hERG channel activity. Interestingly, changing the C-5 substituent on the thiazole ring from Me (**7a**) to CN (**8a**) reduces the hERG liability presumably due to the reduced lipophilic nature of the cyano group.[9]

In an attempt to further improve metabolic stability while retaining JAK2 activity and JAK3 selectivity, we sought to prepare analog **9** (Scheme 3) as it was speculated that the 'naked' C-5 position of the pyrimidine B-ring might constitute another metabolic liability.

The pyrimidine **10** was readily prepared via condensation of urea with methyl fluoromalonate followed by subsequent chlorination of the intermediate 5-fluoropyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione with $POCl_3$.

Compound **9** exhibited good cellular activity (TEL-JAK2 GI_{50} = 0.03 μM) and excellent JAK3 selectivity (TEL-JAK3/TEL-JAK2 = 161-fold). However, rat pharmacokinetic analysis after iv dosing indicated no improvement in the observed clearance (CL = 41 mL/min/kg) compared to **6b**. We therefore reached the conclusion that in rats, the methyl group of the thiazole hinge binder most likely appears to constitute the major metabolic liability.

Despite the less than ideal PK profile of these analogs in rats, compounds **6b**, **7b**, and **9** were administered orally (q.d.) at a dose of 10 mg/kg in nude mice bearing TEL-JAK2 transfected Ba/F3 cells. The percentage (%) inhibition of STAT5 phosphorylation (signal transducers and activators of transcription, PSTAT5) caused by the compounds was determined post-dose at 2 and 6 h. Compound **7b** showed sustained inhibition of PSTAT5 (~50%) 6 h after dosing (Fig. 3). In contrast **6b** only showed substantial inhibition for 2 h after dosing while at the same dose, compound **9** did not appear to prevent the phosphorylation of STAT5 to any appreciable level. The total plasma concentrations of each compound are illustrated in Figure 3. From the data it can be deduced that compound **6b** has a more a profound effect on PSTAT5 inhibition at 2 h (>80%) due to the higher plasma concentration than the other two compounds. For **7b**, the PK/PD correlation likely reflects the excellent potency of this molecule in the TEL-JAK2 proliferation assay. In contrast, **9** did not show any substantial PSTAT5 inhibition presumably by being about sixfold weaker than **7b** in TEL-JAK2 assay although the total plasma concentrations looked similar.

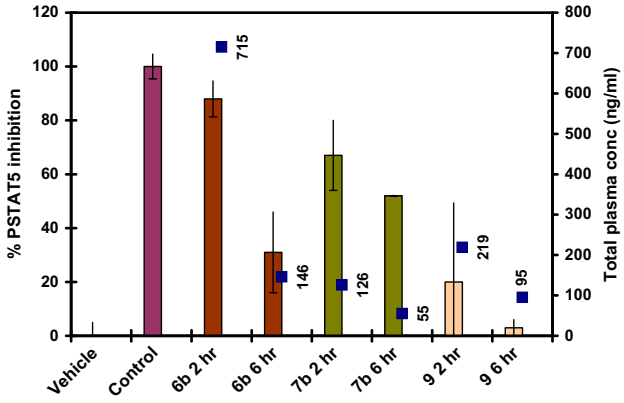
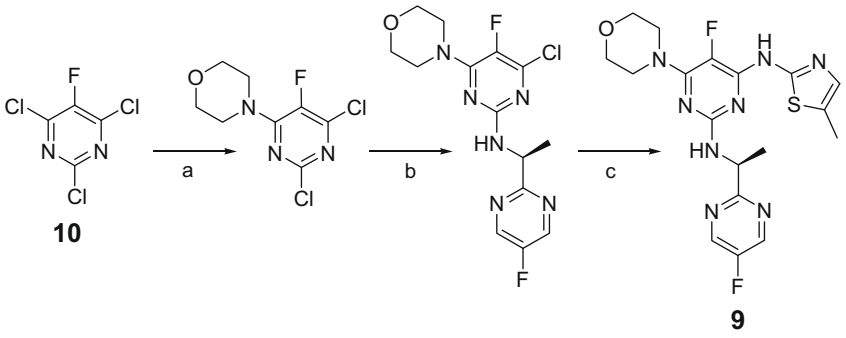


Figure 3. Pharmacodynamic effect of compounds **6b**, **7b**, and **9** on PSTAT5 in the Ba/F3 TEL-JAK2 mouse model and relationship with plasma pharmacokinetics. The bars represent the average percentage (%) inhibition in phosphorylation of STAT5 (±SD, $n = 3$ for each dose and each time point) and are calculated using vehicle and control inhibitor for maximum and minimum value estimation. Nude mice (female) were implanted intravenously with Ba/F3 TEL-JAK2 cells on day 0 and were dosed on day 10. The squares with numbers represent the corresponding average total plasma drug concentration. Mice used in these studies were maintained under specific pathogen-free conditions and were used in compliance with protocols approved by the Institutional Animal Care and Use Committees of AstraZeneca, which conform to institutional and national regulatory standards on experimental animal usage. The pharmacokinetic samples were run on an Applied Biosystems API4000 QTrap using Analyst® software version 1.4.2. The sample analyses were carried out in Sciex#5 LC–MS, using an ACE 3C18 3 μm 20 × 2.1 mm column at ambient temperature and the following eluents: Mobile Phase A: water + 0.1% formic acid + 10 mM ammonium formate B: MeCN + 0.1% formic acid.



Scheme 3. Reagents and conditions: (a) morpholine, EtOH, −20 °C to 0 °C; (b) (*S*)-5-fluoropyrimidin-2-yl-ethanamine hydrochloride (1), Hünig's base, *n*-BuOH, 120 °C; (c) 5-methyl-1,3-thiazol-2-amine, Cs_2CO_3, $Pd_2(dba)_3$, BINAP, dioxane, 95 °C.

In conclusion, we have demonstrated that thiazol-2-yl amine can effectively replace the pyrazol-3-yl amine, leading to potent JAK2 ATP-competitive inhibitors such as **6b**, **7a**–**b**, and **9**. The introduction of solvent tail substituents, such as morpholine, resulted in an improvement in cellular activity. Likewise significant selectivity against JAK3 at the cellular level was observed in some compounds containing solvent tails. However, the moderate pharmacokinetic properties of these compounds in rodents has limited their in vivo activity as shown by the results from testing in the reported pharmacodynamic model.

Acknowledgments

The authors would like to thank Ethan Hoffmann for PK studies and Jing Dai for PK samples analysis. Special thanks to Susan Ashwell and John Haberman for useful recommendations during the preparation of this Letter.

References and notes

1. Gozgit, J. M.; Bebernitz, G.; Patil, P.; Ye, M.; Wu, J.; Su, N.; Wang, T.; Ioannidis, S.; Davies, A. M.; Huszar, D.; Zinda, M. *J. Biol. Chem.* **2008**, *283*, 32334.
2. Ioannidis, S.; Lamb, M. L.; Davies, A. M.; Almeida, L.; Su, M.; Bebernitz, G.; Ye, M.; Bell, K.; Alimzhanov, M.; Zinda, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6524.
3. Wang, T.; Lamb, M. L.; Scott, D. A.; Wang, H.; Block, M. H.; Lyne, P. D.; Lee, J. W.; Davies, A. M.; Zhang, H.; Zhu, Y.; Gu, F.; Han, Y.; Wang, B.; Mohr, P. J.; Kaus, R. J.; Josey, J. A.; Hoffmann, E.; Thress, K.; MacIntyre, T.; Wang, H.; Omer, C. A.; Yu, D. *J. Med. Chem.* **2008**, *51*, 4672.
4. Pierce, A. C.; ter Haar, E.; Binch, A. M.; Kay, D. P.; Patel, S. R.; Li, P. *J. Med. Chem.* **2005**, *48*, 1278.
5. Pierce, A. C.; Sandretto, K. L.; Bemis, G. W. *Proteins: Structure, Function, and Genetics* **2002**, *49*, 567.
6. Das, J.; Chen, P.; Norris, D.; Padmanabha, R.; Lin, J.; Moquin, R. V.; Shen, Z.; Cook, L. S.; Doweyko, A. M.; Pitt, S.; Pang, S.; Shen, D. R.; Fang, O.; de Fex, H. F.; McIntyre, K. W.; Shuster, D. J.; Gillooly, K. M.; Behnia, K.; Schieven, G. L.; Wityak, J.; Barrish, J. C. *J. Med. Chem.* **2006**, *49*, 6819.
7. *Procedure*: To a solution of a mixture of 2-(*E* and *Z*)-3-methoxyacrylonitrile (0.18 mmol, 1.51 mL) in MeCN (3 ml) was added bromine at 0 °C (~1 mL, 0.18 mmol). The resulting mixture was stirred at this temperature for 30 min, whereupon cold water (12 ml) was added. After vigorous stirring for 1 h, $NaOAc{\cdot}3H_2O$ (16 mmol) was added and stirred for 15 min. Thiourea (19.8 mmol) was added and the solution was stirred for 2 h at 0 °C, additional $NaOAc{\cdot}3H_2O$ (10.8 mmol) was added and the resulting mixture was heated to 60 °C for 1 h. The mixture was allowed to cool to room temperature and stirred overnight at this temperature. 10 N NaOH (aq) was added slowly into the reaction mixture until the pH ~4. 2-Aminothiazole-5-carbonitrile was collected by filtration after being washed with cold H_2O. LC–MS: 126 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.10 (s, 2H), 7.81 (s, 1H).
8. Schmid, J.; Busch, U.; Trummlitz, G.; Prox, A.; Kaschke, S.; Waschsmuth, H. *Xenobiotica* **1995**, *25*, 1219.
9. Bilodeau, M. T.; Balitza, A. E.; Koester, T. J.; Manley, P. J.; Rodman, L. D.; Buser-Doepner, C.; Coll, K. E.; Fernandes, C.; Gibbs, J. B.; Heimbrook, D. C.; Huckle, W. R.; Kohl, N.; Lynch, J. J.; Mao, X.; McFall, R. C.; McLoughlin, D.; Miller-Stein, C. M.; Rickert, K. W.; Sepp-Lorenzino, L.; Shipman, J. M.; Subramanian, R.; Thomas, K. A.; Wong, B. K.; Yu, S.; Hartman, G. D. *J. Med. Chem.* **2004**, *47*, 6819.