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ΔF508-CFTR correctors: Synthesis and evaluation of thiazole-tethered imidazolones, oxazoles, oxadiazoles, and thiadiazoles

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ABSTRACT

The most common mutation causing cystic fibrosis (CF) is deletion of phenylalanine residue 508 in the cystic fibrosis transmembrane regulator conductance (CFTR) protein. Small molecules that are able to correct the misfolding of defective ΔF508-CFTR have considerable promise for therapy. Reported here are the design, preparation, and evaluation of five more hydrophilic bisazole analogs of previously identified bithiazole CF corrector **1**. Interestingly, bisazole ΔF508-CFTR corrector activity was not increased by incorporation of more H-bond acceptors (O or N), but correlated best with the overall bisazole molecular geometry. The structure activity data, together with molecular modeling, suggested that active bisazole correctors adopt a U-shaped conformation, and that corrector activity depends on the molecule's ability to access this molecular geometry.

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator protein (CFTR). The most common CFTR mutation is deletion of a phenylalanine residue at position 508, ΔF508, which results in a misfolded CFTR that is retained at the endoplasmic reticulum and rapidly degraded.[1] Failure to provide functioning CFTR, an ATP-gated chloride channel, in epithelial cells in the lungs, pancreas, and other tissues leads to impaired chloride transport. Defective chloride transport results in bacterial growth in the lung due to the accumulation of viscous mucus, and pancreatic malfunction.[2] Intensive efforts were taken to discover small molecules that are able to correct the folding of the ΔF508-CFTR and restore its normal processing and ion channel function[3] with two investigational correctors currently in clinical trial.[4] The recent work of Yoo et al. indicates that bithiazole **1**, a 4-(thiazol-5-yl)thiazole derivative, has significant corrector action on the mutant ΔF508-CFTR.[5] A bithiazole-focused structure–activity-relationship study by Yu et al. showed that an *s-cis* coplanar conformation of the bithiazole, a peripheral pivaloyl group, and a substituted aniline moiety are crucial structural features in eliciting ΔF508-CFTR corrector activity with the bithiazole chemotype.[6] Indeed, the *s-cis* coplanar conformation, as exemplified by bithiazole **1a** wherein the two thiazole rings are constrained by an alkyl chain, is required for corrector activity.[6,7]

However, bithiazoles are relatively hydrophobic (*c*Log*P* = 5.60 and 6.35 for **1** and **1a**, respectively; Fig. 1) and so may not be suitable for development of an orally administered drug (cf., Lipinski's 'rule of five').[8] Considering that *c*Log*P* values for oxazole, oxadiazole, and thiadiazole are much lower (−0.18, −1.41, and −0.60, respectively) than that of a thiazole ring (0.49), we set out to replace one of the two thiazole rings in **1** with these more hydrophilic five-membered aromatic heterocycles to explore different chemotypes in the hope of identifying new CFTR correctors (e.g., **2**) with less hydrophobicity. Given the importance of the peripheral pivalamide and 5-chloro-2-methoxyphenylamine moieties in conveying corrector activity, these two structural motifs were retained in the design of these new bisazole correctors. This ring replacement strategy, in conjunction with considering the relative accessibility of each target molecule, led to a series of bisazole analogs—the *c*Log*P* for which is lowered into the range of 3.95 to 5.20 (Table 1). In addition to increased hydrophilicity, another possible benefit associated with oxygen and nitrogen-rich bisazole rings might be increased and stronger H-bonding interaction within the ΔF508-CFTR binding site. Herein, we report the synthesis of thiazole-tethered imidazolone, oxazole, oxadiazole and thiadiazole bisazole analogs, their ΔF508-CFTR corrector activity, and structure–activity-relationships in this series of bisazoles.

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0960-894X/

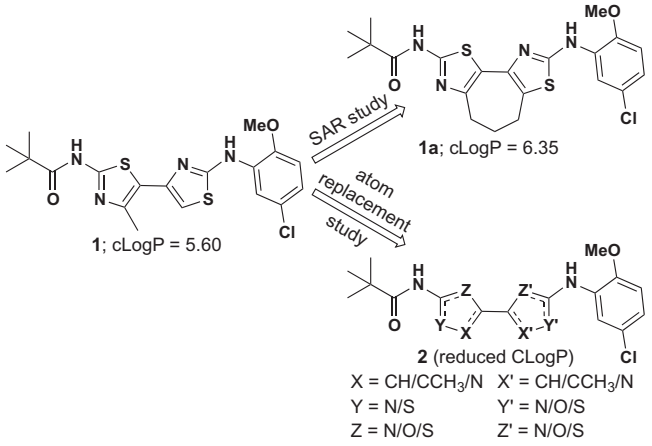
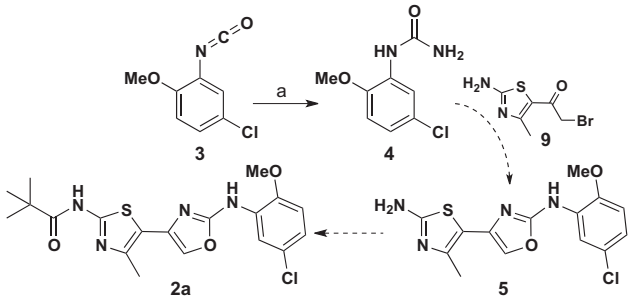


Figure 1. *c*Log*P* and CFTR corrector lead development.

While some directly-linked bisazoles have been reported,[9–11] the thiazole-tethered bisazoles (**2**) targeted here have not been reported in the literature. Indeed, the preparation of directly-linked bisazoles with amide substituents are more difficult than expected as azole formation is problematic in the presence of interfering amino functionalities. With this backdrop, our first target, oxazolyl analog **2a**, was initially expected to be available as depicted in Scheme 1 where isocyanate **3** is converted to urea **4** which would then be condensed with 1-(2-amino-4-methylthiazol-5-yl)-2-bromoethanone to deliver thiazole-oxazole **5**. Chemoselective N-acylation with pivaloyl chloride would then give **2a**. An advantage of this planned route would be the opportunity to incorporate different acid chlorides in this final step. Unfortunately, all attempts at formation of oxazole **5** from urea **4** and thiazole **9** by reference methods were unsuccessful.[12]

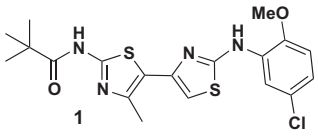
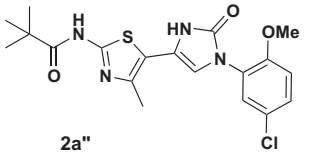
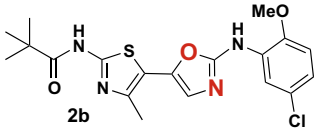
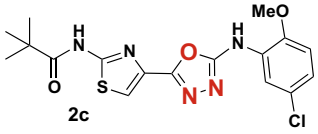
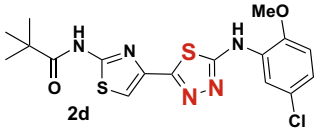
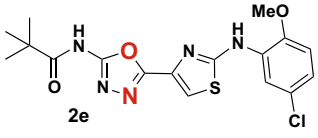
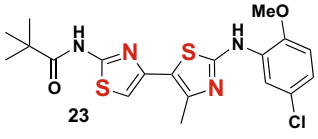


Scheme 1. Reagents & conditions: (a) NH_3, THF, room temperature; 81%.

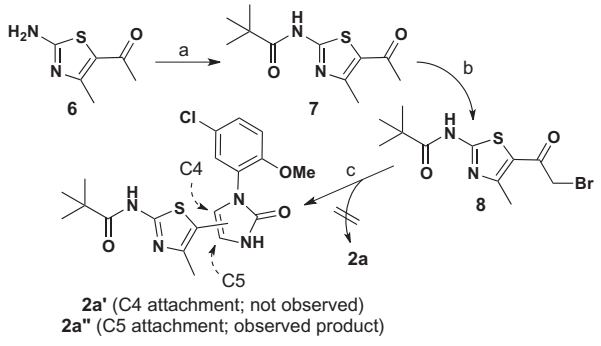
These failed oxazole-forming reactions (**4** + **9** → **5**) prompted us to investigate the alternative route to 4-(thiazol-5-yl)oxazole **2a** detailed in Scheme 2. In this approach, the amino group of thiazole **6** was N-acylated first to give pivalamide **7** in a CDI-mediated coupling reaction (83% yield). We reasoned that making the amide first would decrease electron-donation into the thiazole ring (e.g., **6** vs **7**), thereby increasing the reactivity of the corresponding α-bromoketone **8** with urea **4**. Unfortunately, while bromination of **7** with pyridinium tribromide gave **8** in 90% yield, subsequent reaction of this α-bromo ketone with urea **4** delivered 4-(thiazol-5-yl)-1*H*-imidazol-2(3*H*)-one **2a″** in 18% yield, instead of the targeted 4-(thiazol-5-yl)oxazole **2a**.[13] The Crank/Khan protocol for oxazole formation (replacing the –Br of **8** with an –OH and subsequent reaction with cyanoamide)[14] also did not yield the desired oxazole.

We next set out to prepare oxazole **2b**—the C5 analog of **2a**. This thiazole-tethered oxazole analog was prepared via the three transformations depicted in Scheme 3. Treatment of bromide **9** with sodium azide gave **10** in 78% yield. Subsequent Staudinger reduction of this azide delivered the corresponding α-amino ketone as an unisolated intermediate; addition of thioisocyanate gave a urea intermediate which underwent in situ heterocyclization to give 5-(thiazol-5-yl)oxazole **11** in 44% overall yield. Acylation of **11** with pivaloyl chloride gave **2b** (40% yield).

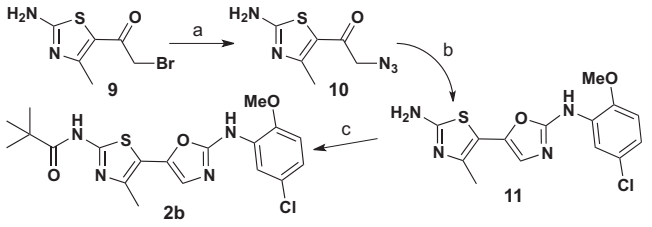
Table 1
Corrector activity, V_{max}, and *c*Log*P* data for bisazoles

 1	EC_{50} (μM)[a] = 0.87 V_{max} (μM/s)[b] = 97.97 *c*Log*P* = 5.60
 2a″	EC_{50} (μM)[a] = not active V_{max} (μM/s)[b] = not active *c*Log*P* = 3.95
 2b	EC_{50} (μM)[a] = 0.74 V_{max} (μM/s)[b] = 84.46 *c*Log*P* = 5.20
 2c	EC_{50} (μM)[a] = 0.66 V_{max} (μM/s)[b] = 65.00 *c*Log*P* = 4.39
 2d	EC_{50} (μM)[a] = not active V_{max} (μM/s)[b] = not active *c*Log*P* = 5.04
 2e	EC_{50} (μM)[a] = 0.59 V_{max} (μM/s)[b] = 35.46 *c*Log*P* = 4.39
 23	EC_{50} (μM)[a] = not active V_{max} (μM/s)[b] = not active *c*Log*P* = 5.60

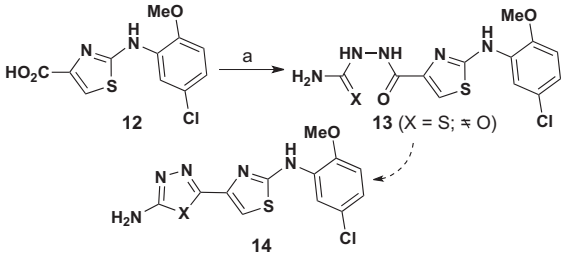
[a] Concentration where the increased I^- influx is 50% of V_{max}.
[b] V_{max} is the maximum increase in I^- influx due to compound dosing.



Scheme 2. Reagents & conditions: (a) pivalic acid, CDI, DMF, 80 °C; 83%. (b) Pyridinium tribromide, 33% HBr in HOAc; 90%. (c) **4**, $CaCO_3$, 120 °C; 18%.



Scheme 3. Reagents and conditions: (a) NaN_3, MeOH, 78%. (b) PPh_3, DCM; 4-chloro-2-isothiocyanato-1-methoxybenzene, DCM, 44%. (c) Pivaloyl chloride, Et_3N, DCM, 40%.



Scheme 4. Reagents & conditions: (a) $NH_2NHC(X)NH_2$ (X = O or S), $EtN(^iPr)_2$, DCC, CH_2Cl_2.

Our initial approach to oxadiazole and thiadiazole analogs is shown in Scheme 4, but two problems were encountered with this route. Firstly, thiazole-4-carboxylic **12** reacted effectively with hydrazinecarbothioamide (→ **13**, X = S), but not hydrazinecarboxamide (→ **13**, X = O). Secondly, carbothioamide **13** (X = S) could not be cyclized to thiadiazole **14** (X = S) in a practical yield under any of the protocols reported in literature.[15]

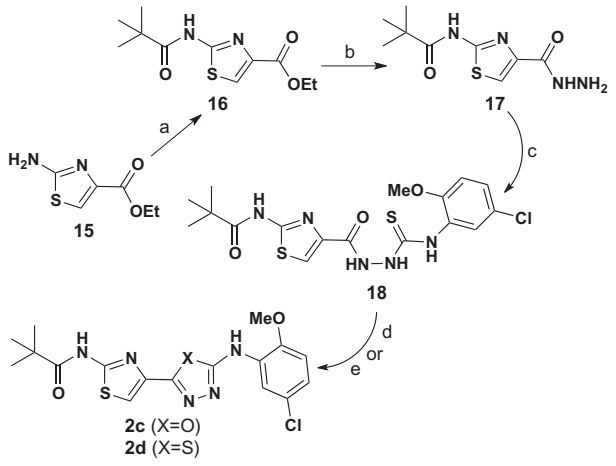
In light of these issues, we decided to focus on replacing the right-hand thiazole ring of lead compound **1** (see Fig. 1) with oxadiazole and thiadiazole heterocycles. Both targeted analogs, **2c** and **2d**, were delivered via the unified route depicted in Scheme 5. In this approach, 2-aminothiazole **15** was converted to amide **16**, which was then reacted with hydrazine hydrate to give thiazole-4-carbohydrazide **17** in 60% overall yield from **15**. Hydrazide **17** was next reacted with 4-chloro-2-isothiocyanato-1-methoxybenzene to give 2-carbonylhydrazinecarbothioamide **18**. From this common intermediate, 2-(thiazol-4-yl)-1,3,4-oxadiazole **2c** was obtained in 66% yield by the reaction of **18** with tosyl chloride in pyridine, while 2-(thiazol-4-yl)-1,3,4-thiadiazole **2d** was accessed via a phosphoryl trichloride-mediated cyclization process in 75% yield.

Finally, 2-(thiazol-4-yl)-1,3,4-oxadiazole analog **2e** was prepared via the four-step process shown in Scheme 6. Treatment of N-substituted thiourea **19** with ethyl 3-bromo-2-oxopropanoate produced ethyl thiazolo-4-carboxylate **20** in 92% yield. Reacting ester **20** with hydrazine hydrate produced **21** and treatment of this hydrazide with CNBr delivered 1,3,4-oxadiazole **22** in 60% yield. Microwave irradiation of this 1,3,4-oxadiazol-2-amine with pivaloyl chloride in 1,4-dioxane (+TEA) gave **2e** in 45% yield.

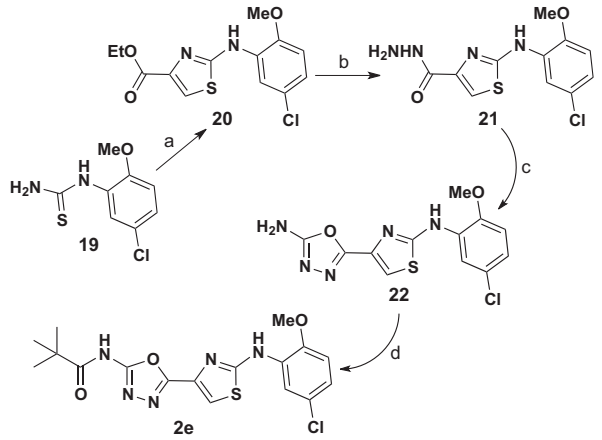
Bisazole analogs **2a**″/**2b**–**e** were assayed for ΔF508-CFTR correct- or activity using our well-established cell-based corrector assay. Briefly, the influx of I^- as surrogate for Cl^- was measured in FRT cells co-expressing human ΔF508-CFTR employing the I^--sensitive fluorescent sensor YFP-H148Q/I152L.[16,17] Following 24 h incubation with each test compound, I^- influx was determined from the kinetics of YFP-H148Q/I152L quenching in response to I^- addition in cells treated with a cAMP agonist and the potentiator genistein. The corrector activity of each analog was calculated from influx versus concentration data.[18] The corrector activity of bisazole analogs **2a**″/**2b–e**, as well as lead compound **1** and reference bithiazole compound **23**,[2] are listed in Table 1.

Three of the five new bisazole analogs, thiazole-tethered oxazole **2b** and oxadiazoles **2c** and **2e**, are effective at recovering the ion efflux function of ΔF508-CFTR. Among these three active bisazoles, 5-(oxazol-5′-yl)oxazole analog **2b** is more potent than the oxadiazolyl analogs (**2c** and **2e**) and its corrector activity is comparable with lead compound **1**. Interestingly, thiadiazole analog **2d** and heteroatom invertomer **23** are not active. Thiazole-tethered oxazole **2b** is active in the corrector assay, while, not surprisingly, thiazole-tethered imidazol-2-one **2a**″ is not. It is intriguing to note that the active oxazole analog **2b** is an isostere of inactive bithiazole **23**. Collectively, the results within this series suggest that corrector activity is tied to overall molecular geometry.

Conformational searches on **1**, **2a–e** and **23** were carried out using the Merck molecular force field in Spartan.[19,20] The lowest energy conformers were then optimized using GAUSSIAN09[21] with the M06-2X/6-31+G(d,p)[22] density functional method. Relative energies were calculated both in the gas phase (as an extreme model of nonpolar environments) and water (using the SMD continuum solvation method).[23] Structural drawings in Table 2 were produced using CYLView.[24]

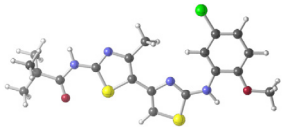
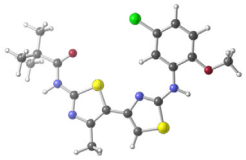
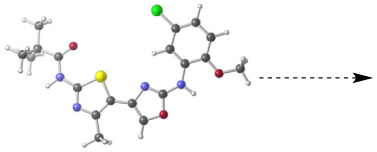
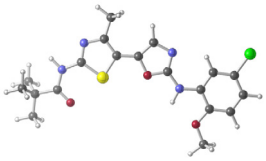
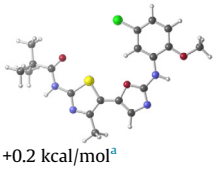
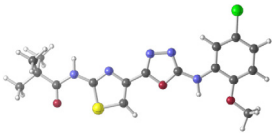
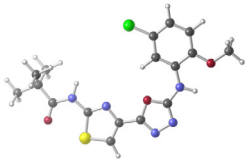
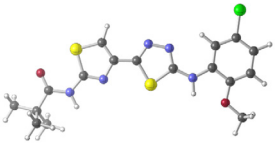
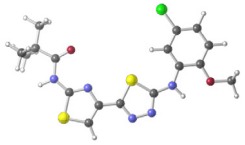
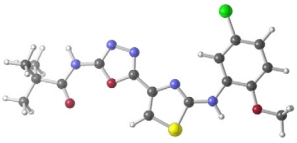
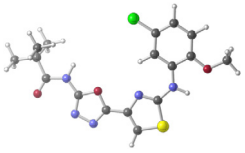
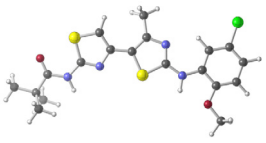
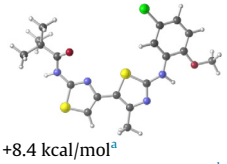


Scheme 5. Reagents & conditions: (a) pivaloyl chloride, DCM, TEA, 50 °C, 1 h; 85%. (b) $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 5 h; 71%. (c) 4-Chloro-2-isothiocyanato-1-methoxybenzene, THF, rt, 18 h; 88%. (d) (→ **2c**) TsCl, THF, pyridine, 75 °C, 3 h; 66%. (e) (→ **2d**) $POCl_3$, 110 °C, 4 h; 75%.



Scheme 6. Reagents & conditions: (a) ethyl 3-bromo-2-oxopropanoate, ethanol, 90 °C, 3 h; 92%; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, 90 °C, 12 h; 77%; (c) CNBr, MeOH, rt, 18 h; 60%; (d) pivaloyl chloride, 1,4-dioxane, TEA, microwave, 40 min; 45%.

Table 2
Results of conformational analyses

Compd	Active or inactive	Lowest energy conformer in water	Lowest energy U-shaped conformer in water
1	Active		 +7.1 kcal/mol[a] −0.2 kcal/mol in gas phase[b]
2a	Unknown (see **2a**″)		same
2b	Active		 +0.2 kcal/mol[a] −1.3 kcal/mol in gas phase[b]
2c	Active		 +1.7 kcal/mol[a] +0.6 kcal/mol in gas phase[b]
2d	Inactive		 +7.3 kcal/mol[a] +12.2 kcal/mol in gas phase[b]
2e	Active		 +1.0 kcal/mol[a] −0.9 kcal/mol in gas phase[b]
23	Inactive		 +8.4 kcal/mol[a] +5.9 kcal/mol in gas phase[b]

[a] Free energies are relative to the lowest energy conformer in water.
[b] Free energies are relative to the lowest energy conformer in the gas phase.

Our previous work,[5–7] especially that with tethered bithiazoles such as **1a**, provides circumstantial evidence in support of a 'U-shaped' bioactive conformation (Fig. 2), rigidified in part by S···lone pair interactions.[6,25] The overall lowest energy conformer found for each compound, along with the lowest energy U-shaped conformer (both in water) are shown in Table 2 (see Supporting information for additional details, including interatomic distances). These results indicate that: (1) the preferred conformer varies from bisazole to bisazole and (2) not having a U-shaped conformer as the preferred conformation does not preclude activity (see **1** and **2b**,**c**,**e**) when a U-shaped conformer can be readily accessed (i.e., gas phase U-shaped conformer within ~1 kcal/mol of the lowest energy conformer; that activity appears to track with gas phase energies suggests that the target binding site is not particularly polar). Of course, having an accessible U-shaped conformer does not guarantee activity, since a compound could be inactive due to subtle differences in non-covalent interactions with its binding site, off-target effects or other issues with this whole cell assay. Point (2) above suggests that the putative bioactive conformation shown in Figure 2 likely needs to be accessible for

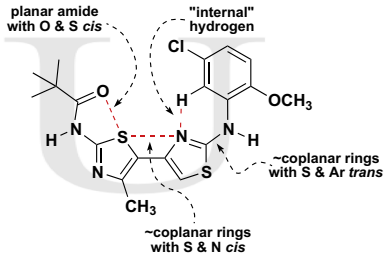


Figure 2. Putative bioactive U-shaped conformation (illustrated for bithiazole **1**).

activity; note that gas phase U-shaped conformers for inactive **2d** and **23** are 12.2 and 5.9 kcal/mol, respectively, higher in energy than their preferred conformations. Our previous work indicates that the conformation about the inter-ring bond (see ~coplanar bisazole rings in Fig. 2) correlates with activity as constrained **1a** is more active than unconstrained **1**.[5–7] It therefore appears that access to the conformational control elements highlighted in Figure 2 are required for activity.

Thiazole-tethered bisazoles [thiazole-oxazole (**2b**), thiazole-oxadiazole (**2c** and **2e**), and thiazole-thiadiazole (**2d**)], which are structurally related to the bithiazole ΔF508-CFTR corrector **1**, were prepared from carbonyl-substituted thiazoles and a series of new bisazole CF correctors have been identified. While the newly identified bisazole correctors contain more oxygen and/or nitrogen atoms and have lower *c*Log*P* values than bithiazole **1**, their ΔF508-CFTR corrector activity does not correlate with the number of the H-bonding acceptors. SAR analysis suggested that incorporation of more H-bond acceptors can fundamentally change the molecular geometry in these bisazole systems (see **1** vs **2b–e** vs **23**). Finally, bithiazole-to-bisazole atom substitution can modulate the accessibility of the U-shaped conformation required for corrector activity.

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Supplementary data

Supplementary data (experimental details) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.067.

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13. (a) The authors thank BMCL Reviewer #1 for thoughtful comments regarding Scheme 1 and **2a** versus **2a′**/**2a″**. Using NMR chemical shift calculations {done using the GIAO[13b–f] method at the SCRF-mPW1PW91/6-311+G(2d,p)//M06-2X/6-31+G(d,p) level[13g] and empirically scaled [SCRF refers to the inclusion of solvent effects (chloroform) using the SMD continuum model][13h,13i]}, structures **2a** and **2a′** are ruled out as the product of the reaction of **8** + **4** and **2a″** is assigned as the product (see SI for details). Mean absolute deviations were 0.4, 0.4, and 0.1 for ^{1}H NMR and 4.6, 3.0, and 3.1 for ^{13}C NMR (**2a**, **2a′**, and **2a″**, respectively). Furthermore, DP4 analysis[13j] showed 0.4% and 98.7% match for ^{1}H NMR and 33.6% and 66.4% match for ^{13}C NMR to experimental chemical shifts (**2a′** and **2a″**, respectively); **2a″** is therefore assigned as the **8** + **4** product.; (b) London, F. *J. Phys. Radium* **1937**, *8*, 397; (c) McWeeny, R. *Phys. Rev.* **1962**, *126*, 1028; (d) Ditchfield, R. *Mol. Phys.* **1974**, *27*, 789; (e) Wolinski, K.; Hilton, J. F.; Pulay, P. *J. Am. Chem. Soc.* **1990**, *112*, 8251; (f) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J. *J. Chem. Phys.* **1996**, *104*, 5497; (g) Matsuda, S. P. T.; Wilson, W. K.; Xiong, Q. *Org. Biomol. Chem.* **2006**, *4*, 530; (h) Lodewyk, M.; Siebert, M. R.; Tantillo, D. *Chem. Rev.* **1839**, *2012*, 112; (i) Frisch, M. J. Gaussian 09 (revision B.01); see SI for full author list.; (j) Smith, S. G.; Goodman, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 12946.
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