JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Stereoelectronic Effects Dictate Molecular Conformation and Biological Function of Heterocyclic Amides

Robert C. Reid,* Mei-Kwan Yau, Ranee Singh,† Junxian Lim, and David P. Fairlie*

Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072, Australia

Supporting Information

ABSTRACT: Heterocycles adjacent to amides can have important influences on molecular conformation due to stereoelectronic effects exerted by the heteroatom. This was shown for imidazole- and thiazole-amides by comparing low energy conformations (ab initio MP2 and DFT calculations), charge distribution, dipole moments, and known crystal structures which support a general principle. Switching a heteroatom from nitrogen to sulfur altered the amide conformation, producing different three-dimensional electrostatic surfaces. Differences were attributed to different dipole and orbital alignments and spectacularly translated into opposing agonist vs antagonist functions in modulating a G-protein coupled receptor for inflammatory protein complement C3a on human macrophages. Influences of the heteroatom were confirmed by locking the amide conformation using fused bicyclic rings. These findings show that stereoelectronic effects of heterocycles modulate molecular conformation and can impart strikingly different biological properties.

Heterocyclic rings are important components of many organic compounds, including natural products, pharmaceuticals, and peptidomimetics, and can influence molecular conformation, solubility, chemical and biological activity.[1] Heteroatoms are hydrogen bond donors or acceptors, general acids or bases, and confer different electrostatic properties that fine-tune chemical reactivity or biological interactions.[2] Heterocycles can constrain molecular conformation by orienting substituents or through intramolecular interactions.[3] For example, heterocycles such as the imidazole in **1** and the thiazole in **2** (Figure 1) might influence the conformation of the adjacent amide due to 1,4-$N_{(heteroatom)}\cdots O_{(amide)}$ or 1,4-$S_{(heteroatom)}\cdots O_{(amide)}$ orbital interactions.

Ab initio calculations (Figure 1a) suggest that the trigonal nitrogen of the imidazole adjacent to the carbonyl of **1** may have a large partial negative charge, whereas the sulfur atom in the aromatic thiazole ring may carry a partial positive electrostatic potential. The electronegative carbonyl oxygen adjacent to the thiazole in **2** should preferentially adopt a cis orientation due to a significant electrostatic attraction. Noncovalent $n \rightarrow \sigma^*$ interactions between lone pairs and electron deficient sulfur have been described previously[5] and associated with drug-like molecules.[6] Ab initio calculations for **2** suggest that the S−O distance (2.93 Å) is less than the sum of the sulfur and oxygen van der Waals radii (3.4 Å),[7] supporting a noncovalent interaction. Conversely, compound **1** should adopt a trans orientation due to electron lone pair repulsion separating the negatively charged atoms.[8] A significant barrier to rotation was predicted to stabilize these different conformations. To assess this, we performed ab initio density functional theory (DFT) calculations at the B3LYP/6-311++G(2d,2p) level of theory on **1** and **2** to identify conformational preferences (Figure 1b). The dihedral angle X−C−C−O was varied in 10° increments with subsequent energy minimization, and the total energy of each conformer was plotted relative to the minimum energy structure. All calculations were performed using Gaussian 09 through the graphical interface GaussView 5.[4]

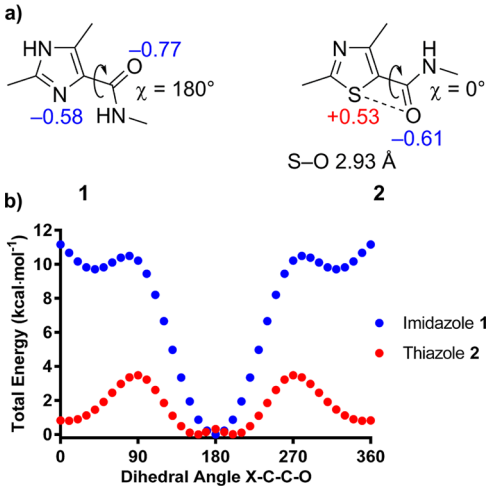


Figure 1. (a) Partial charges on heteroatoms determined by natural population analysis on geometries optimized at MP2/6-311++G(2d,2p) level using Gaussian 09.[4] Dihedral angle (χ); S−O distance (Å). (b) Variation in energy with dihedral angle X−C−C−O reveals barriers to rotation from low energy conformations. 1*H*-Imidazole-4-carboxamide **1** (blue) shows preferred dihedral angle 180° with high barrier to rotation. Thiazole-5-carboxamide **2** (red) has access to low energy conformers with dihedral angles close to 0° and 180°.

The imidazole carboxamide **1** had a strong preference for a dihedral angle N−C−C−O ~180° with a high barrier to rotation (10.5 kcal·mol^{-1}) to a conformer with dihedral angle 40° and 9.7 kcal·mol^{-1} higher in energy than the lowest energy conformer. By contrast, the thiazole carboxamide **2** had no

Received: July 2, 2014
Published: August 7, 2014

   dx.doi.org/10.1021/ja506518t | J. Am. Chem. Soc. 2014, 136, 11914−11917

preference for either conformation ($\Delta E \sim 0.8$ kcal·mol^{-1}), and only a small barrier to rotation (~3.5 kcal·mol^{-1}). The fractional population of conformers of different energy followed a Boltzmann distribution, with ~100% of 1*H*-imidazole-4-carboxamides having a dihedral angle N−C−C−O of 180°, and rotation to alternate conformations being energetically unfavorable.

These conformational preferences are consistent with the expected alignment of the electric dipole moments of the heterocyclic ring and carbonyl group connected by a rotatable bond. Figure 2a shows that the dipole moment of imidazole

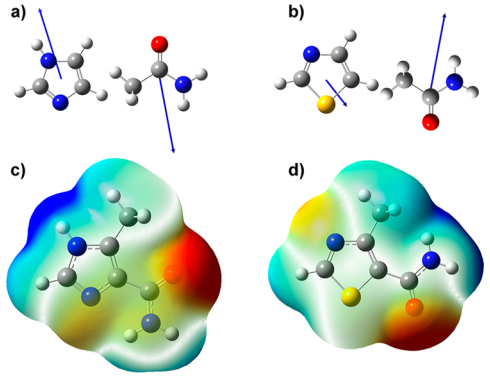


Figure 2. Electric dipole moment of heterocycles and amide carbonyl depicted by vectors (blue arrows) with magnitude proportional to length (3 D·cm^{-1}) and direction toward $\delta+$ (arrowhead). Favored antiparallel dipoles require different dihedral angles: (a) 1*H*-imidazole-4-carboxamide N−C−C−O dihedral angle 180°; (b) thiazole-5-carboxamide S−C−C−O dihedral angle 0°. Electrostatic surface potential: (c) 1*H*-imidazole-4-carboxamide **1**, (d) thiazole-5-carboxamide **2**. Calculations used Gaussian 09, images in GaussView 5.[4]

(3.88 D) is oriented away from the trigonal nitrogen atom at the 3-position, thus an appended carbonyl group (4.28 D for acetamide) must adopt a dihedral angle N−C−C−O of 180° for these dipoles to align in preferred antiparallel directions. Conversely, the weaker dipole moment of thiazole (1.65 D) points in the opposite direction, away from the trigonal nitrogen at the 3-position. When an amide carbonyl group is attached, it preferentially adopts an S−C−C−O dihedral angle ~0° to place these dipoles in antiparallel directions, although the effect is weaker (Figure 2b). These differences result in different electrostatic surfaces (Figure 2c,d) that are predicted to result in different chemical and biological properties.

To test these conformational predictions, we used ConQuest to search the Cambridge Structural Database[9] (v5.34, 2012) for crystal structures containing imidazole-, thiazole- and thiophene-carboxamides (Figure 3, Supplementary Tables 1−3). All 34 diverse structures for 1*H*-imidazole-4-carboxamides (**3**) showed a conformation with a N−C−C−O dihedral angle 153−180° (ave 173°). Of six thiazole-5-carboxamide analogues

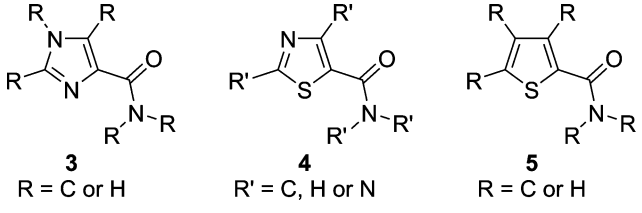


Figure 3. Query structures **3**−**5** searched in the CSD.[9]

(**4**), four showed a S−C−C−O dihedral angle 11−37°, while two had the opposite conformation (165° and 173°). Of 54 thiophene-2-carboxamide compounds (**5**), all but two (163° and 166°) had a S−C−C−O dihedral angle 0−49° (ave 13°). In all structures where S−C−C−O was ≤40°, the S−O distance (2.8−3.0 Å) was less than the sum of the individual sulfur and oxygen van der Waals radii (3.4 Å)[7] and comparable with our calculated value of 2.93 Å (Figure 1a).

This suggests that an attractive nonbonded sulfur−oxygen interaction,[9c,d,10] exists for thiazole amides (e.g., **2**), whereas electron pair repulsion theory[8] predicts that the amide carbonyl oxygen would favor the conformer with dihedral angle N−C−C−O 180° for 1*H*-imidazole-4-carboxamide (e.g., **1**), as observed. Thus, structurally very similar heterocycles do indeed adopt quite different conformations that might translate into very different chemical and biological properties.

On the basis of these findings, we modified a simple flexible ligand (N2-[(2,2-diphenylethoxy)acetyl]-L-arginine), reported to compete with a human inflammatory protein (complement C3a) for a G protein-coupled receptor found on human immune cells.[11] We synthesized compounds **6** and **7** containing a heterocyclic carboxamide, where the only difference was the heterocycle, imidazole **6** versus thiazole **7**. We found that these compounds had completely opposite biological functions in modulating the human complement C3a protein receptor (Figure 4). While **6** potently activated the receptor (i.e., an agonist), **7** inhibited its activation (i.e., an antagonist).

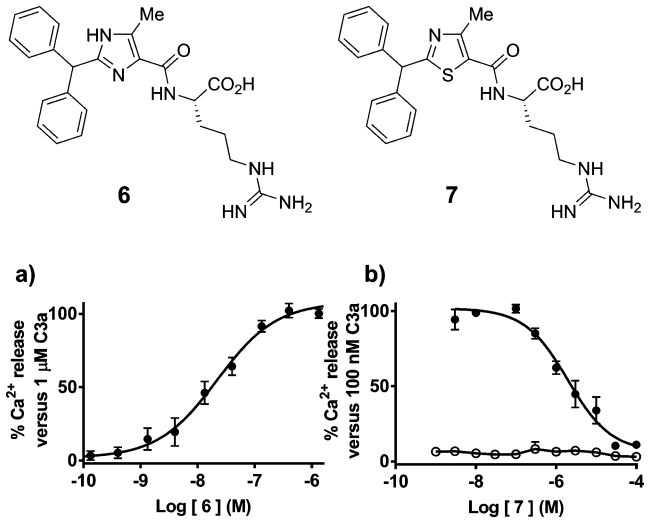


Figure 4. Structures for compounds **6** and **7**. (a) Intracellular Ca^{2+} release in human macrophages induced by different concentrations of agonist **6** (EC_{50} 24 nM) and (b) compound **7** has no agonist activity (○) but increasing concentrations of antagonist **7** (●) (IC_{50} 1 μM) block iCa^{2+} release induced by 100 nM hC3a. All data $n \geq 3$, error bars are ± SEM.

These opposing functions are due to the imidazole and thiazole rings in compounds **6** and **7** promoting two distinctly different conformations controlled by stereoelectronic effects intrinsic to the specific heterocycle present (Figure 1). Although the thiazole can also adopt a conformation with an S−C−C−O dihedral angle ~180°, the receptor preferentially binds its conformer with a dihedral angle ~0° which the imidazole cannot access because of the high barrier to rotation.

This surprising switch in function between compounds **6** and **7** (Figure 4) was not related to the NH present at the 1-

dx.doi.org/10.1021/ja506518t | J. Am. Chem. Soc. 2014, 136, 11914−11917

position of the imidazole (perhaps through a hydrogen bond to the receptor). We synthesized the isomeric thiazole compound **8** and the 1-methyl-1*H*-imidazole analogue **9** which are incapable of making such interactions. Both had similar biological activity (agonists) to the imidazole **6** (Figure 5).

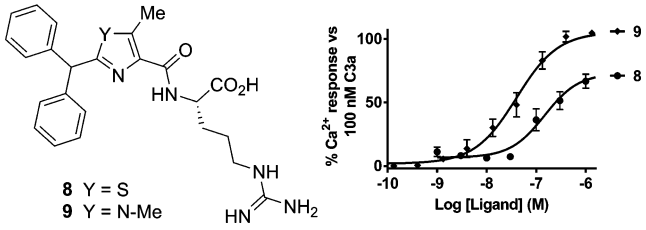


Figure 5. Thiazole **8** and 1-methyl-1*H*-imidazole analogue **9** have agonist activity similar to **6**.

Compound **8** was less effective than **9**, suggesting that fine-tuning of activity was influenced by subtle changes to other regions. Compound **9** also removes any ambiguity about the tautomeric form of the biologically active imidazole **6**. Thus, subtle changes to electrostatic surface potentials (mapped in Figure 2c,d) dramatically alter complementarity between ligand and receptor, conferring a switch in biological function.

To confirm the importance of stereoelectronic effects in dictating conformation in these heterocycle−carboxamide compounds, we designed conformationally rigid compounds **10** and **11**. Since stereoelectronic effects enforce 1*H*-imidazole-4-carboxamides to adopt an N−C−C−O dihedral angle ∼180° as in **1** and hence **6**, we designed and synthesized the constrained 1*H*-imidazo[4,5-*c*]pyridine analogue **10**. This presents a strong hydrogen bond-accepting trigonal nitrogen, and a heterocycle locked into this arrangement. Creating the pyridine ring was not expected to impinge on interactions that **10** may make with the receptor, because the 5-methyl group of **6** shields one of the electron lone pairs on the amide oxygen leaving only one hydrogen bond acceptor, the pyridine trigonal nitrogen of **10**. The arginine αNH proton remains available as a

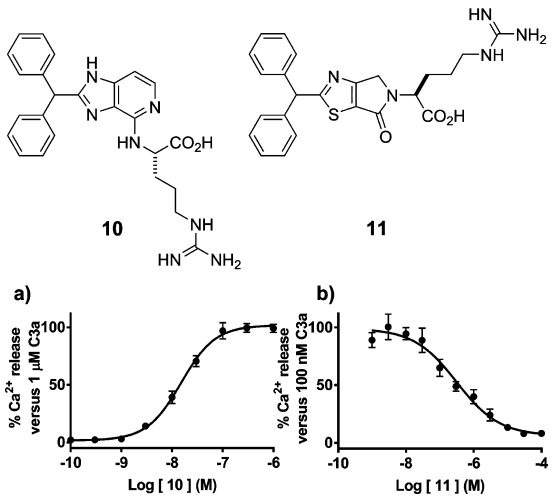
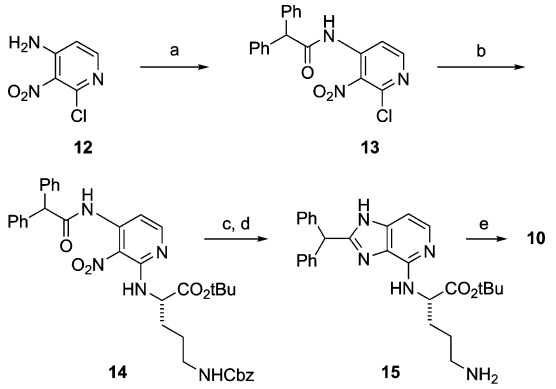


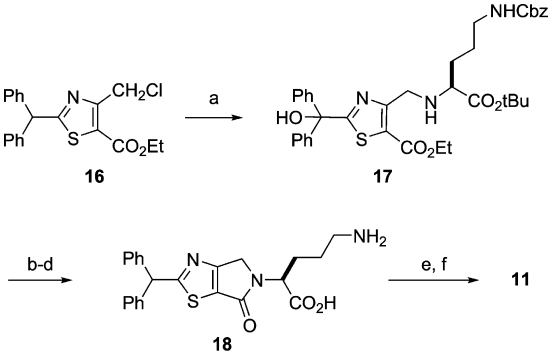
Figure 6. Structures of the constrained 1*H*-imidazo[4,5-*c*]pyridine **10** and lactam **11**: (a) intracellular Ca^{2+} release in macrophages induced by different concentrations of agonist **10** (EC_{50} 15 nM) and (b) increasing concentration of antagonist **11** blocks iCa^{2+} release induced by hC3a (100 nM) (IC_{50} 0.32 μM). All data $n \geq 3$, error bars are ± SEM.

Scheme 1. Synthesis of the Constrained Agonist 10[a]



[a](a) NaO^{t}Bu, $Ph_2CHCOCl$, THF −10 °C; (b) H-Orn(Cbz)-O^{t}Bu, NMM, EtOH, Δ; (c) H_2, 10% Pd−C, EtOH, room temp, 30 min; (d) glacial AcOH, 40 °C, 30 min; (e) *N*,*N*′-Di-Boc-1*H*-pyrazole-1-carboxamidine, DMF; (f) TFA.

Scheme 2. Synthesis of the Constrained Lactam Antagonist 11[a]



[a](a) H-Orn(Cbz)-O^{t}Bu, K_2CO_3, NaI, DMF; (b) NaOH, H_2O, MeOH, THF; (c) DCC, DCM; (d) TFA, Et_3SiH, PhSMe; (e) *N*,*N*′-Di-Boc-1*H*-pyrazole-1-carboxamidine, DMF; (f) TFA.

hydrogen bond donor and thus the hydrogen bonding properties of **10** are expected to resemble those observed in, for example, adenine−thymine DNA base pairing. Compound **10**, prepared via Scheme 1, was an agonist (Figure 6a) just like its more flexible congener **6** (Figure 4).

Support for the hypothesis, that physiological function is driven by ligand conformation, was also sought for the thiazole-5-carboxamide with an S−C−C−O dihedral angle ∼0°, as in compound **2**. The conformationally locked lactam **11** was consequently designed and synthesized via Scheme 2. The methylene group that completes the lactam ring fuses the 4-methyl group and amide nitrogen, thereby restricting the lactam carbonyl to one conformation where the S−C−C−O dihedral angle was close to 0°. The arginine αNH proton is removed as a consequence of lactam formation, but this is unlikely to be important for receptor binding because the amide NH in **7** is shielded from receptor interactions by the 5-methyl group. Compound **11** was found to be an antagonist (Figure 6b) just like its more flexible analogue **7**.

The 1*H*-imidazo[4,5-*c*]pyridine **10** was prepared as shown in Scheme 1. The known 4-amino-3-nitro-2-chloropyridine[12] **12** was acylated with diphenylacetyl chloride after deprotonation with NaO^{t}Bu at −10 °C to give anilide **13**. Nucleophilic aromatic substitution of **13** with *N*δ-Cbz-L-ornithine *tert*-butyl

dx.doi.org/10.1021/ja506518t | J. Am. Chem. Soc. 2014, 136, 11914−11917

ester gave **14**. Hydrogenation reduced the nitro group to amine and simultaneously cleaved the Cbz protecting group. Cyclization of the aminoanilide intermediate to the 1*H*-imidazo[4,5-*c*]pyridine **15** occurred rapidly and cleanly in glacial acetic acid solution with only mild heating. The amino group was converted to the guanidine using *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine, and removal of the *tert*-butyl ester and Boc groups with TFA gave **10**. Lactam **11** was synthesized (Scheme 2) by nucleophilic substitution of chloromethylthiazole **16** with *Nδ*-Cbz-L-ornithine *tert*-butyl ester to give **17**, accompanied by hydroxylation of the benzhydryl group despite effort to exclude moisture and air. This was removed during subsequent deprotections. Hydrolysis of ethyl ester **17** followed by cyclization to lactam, and deprotection including ionic hydrogenation gave **18**. The amino group was converted to the guanidine using *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine and deprotected with TFA to give **11** (Scheme 2, details in Supporting Information).

In summary, multiple approaches used here show that an aromatic heterocycle dictates the conformation of an adjacent carbonyl group. This finding enables predictions of conformational preferences for heterocyclic carboxamides, that can profoundly influence interactions of small molecules with proteins. This analysis is expected to extend to other heterocyclic ring systems, where a single rotatable bond links the heteroatom to a polar substituent. For example, we found >200 picolinamides and twelve 1,2,3-triazole-4-carboxamides in the CSD where the N−C−C−O dihedral angle was ∼180°. DFT calculations reveal that both trans conformers are respectively 9.1 and 8.7 kcal/mol more stable. Understanding orbital and dipole alignments can enable more rational selection of heterocyclic templates for elaboration in drug design and discovery.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, analytical data (^{1}H, ^{13}C NMR) for all new compounds, CSD searches and cell assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

r.reid@imb.uq.edu.au
d.fairlie@imb.uq.edu.au

Present Address

†R.S.: Dept. Pharmacol. & Toxicol., Faculty of Veterinary Medicine, Khon Kaen University, Thailand.

Notes

The authors declare the following competing financial interest(s): The authors are inventors of a patent application describing C3a receptor agonists and antagonists owned by the University of Queensland.

■ ACKNOWLEDGMENTS

This study was supported by grants to DPF from the National Health and Medical Research Council (APP1000745, APP1028423, SPRF fellowship 1027369), the Australian Research Council (DP130100629, DP1093245, FF0668733), the ARC Centre of Excellence in Advanced Molecular Imaging (CE140100011), and the Queensland Government (CIF grant).

■ REFERENCES

(1) (a) Baumann, M.; Baxendale, I. R.; Ley, S. V.; Nikbin, N. *Beilstein J. Org. Chem.* **2011**, *7*, 442. (b) Reid, R. C.; Yau, M.-K.; Singh, R.; Hamidon, J. K.; Reed, A. N.; Chu, P.; Suen, J. Y.; Stoermer, M. J.; Blakeney, J. S.; Lim, J.; Faber, J. M.; Fairlie, D. P. *Nat. Commun.* **2013**, *4*, 2802.

(2) Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529.

(3) Schaerfer, C.; Schulz-Gasch, T.; Ehrlich, H. C.; Guba, W.; Rarey, M.; Stahl, M. *J. Med. Chem.* **2013**, *56*, 2016.

(4) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; *Gaussian 09*, revision A.02; Gaussian Inc.: Wallingford CT, 2009.

(5) (a) Murray, J. S.; Lane, P.; Politzer, P. *Int. J. Quantum Chem.* **2008**, *108*, 2770. (b) Dudkin, V. Y. *Chem. Heterocycl. Compd. (N. Y., NY, U. S.)* **2012**, *48*, 27.

(6) (a) Franchetti, P.; Cappellacci, L.; Grifantini, M.; Barzi, A.; Nocentini, G.; Yang, H.; O'Connor, A.; Jayaram, H. N.; Carrell, C.; Goldstein, B. M. *J. Med. Chem.* **1995**, *38*, 3829. (b) Goldstein, B. M.; Li, H.; Hallows, W. H.; Langs, D. A.; Franchetti, P.; Cappellacci, L.; Grifantini, M. *J. Med. Chem.* **1994**, *37*, 1684. (c) Goldstein, B. M.; Takusagawa, F.; Berman, H. M.; Srivastava, P. C.; Robins, R. K. *J. Am. Chem. Soc.* **1983**, *105*, 7416. (d) Haginoya, N.; Kobayashi, S.; Komoriya, S.; Yoshino, T.; Suzuki, M.; Shimada, T.; Watanabe, K.; Hirokawa, Y.; Furugori, T.; Nagahara, T. *J. Med. Chem.* **2004**, *47*, 5167. (e) Hayashi, K.; Ogawa, S.; Sano, S.; Shiro, M.; Yamaguchi, K.; Sei, Y.; Nagao, Y. *Chem. Pharm. Bull.* **2008**, *56*, 802. (f) Hynes, J., Jr.; Wu, H.; Pitt, S.; Shen, D. R.; Zhang, R.; Schieven, G. L.; Gillooly, K. M.; Shuster, D. J.; Taylor, T. L.; Yang, X.; McIntyre, K. W.; McKinnon, M.; Zhang, H.; Marathe, P. H.; Doweyko, A. M.; Kish, K.; Kiefer, S. E.; Sack, J. S.; Newitt, J. A.; Barrish, J. C.; Dodd, J.; Leftheris, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1762. (g) Ioannidis, S.; Lamb, M. L.; Almeida, L.; Guan, H.; Peng, B.; Bebernitz, G.; Bell, K.; Alimzhanov, M.; Zinda, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1669. (h) Jung, F. H.; Pasquet, G.; Van der Brempt, C. L.; Lohmann, J.-J. M.; Warin, N.; Renaud, F.; Germain, H.; De Savi, C.; Roberts, N.; Johnson, T.; Dousson, C.; Hill, G. B.; Mortlock, A. A.; Heron, N.; Wilkinson, R. W.; Wedge, S. R.; Heaton, S. P.; Odedra, R.; Keen, N. J.; Green, S.; Brown, E.; Thompson, K.; Brightwell, S. *J. Med. Chem.* **2006**, *49*, 955. (i) Li, H.; Hallows, W. A.; Punzi, J. S.; Marquez, V. E.; Carrell, H. L.; Pankiewicz, K. W.; Watanabe, K. A.; Goldstein, B. M. *Biochemistry* **1994**, *33*, 23. (j) Lin, S.; Wrobleski, S. T.; Hynes, J., Jr.; Pitt, S.; Zhang, R.; Fan, Y.; Doweyko, A. M.; Kish, K. F.; Sack, J. S.; Malley, M. F.; Kiefer, S. E.; Newitt, J. A.; McKinnon, M.; Trzaskos, J.; Barrish, J. C.; Dodd, J. H.; Schieven, G. L.; Leftheris, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5864. (k) Nagao, Y.; Hirata, T.; Goto, S.; Sano, S.; Kakehi, A.; Iizuka, K.; Shiro, M. *J. Am. Chem. Soc.* **1998**, *120*, 3104. (l) Pierce, A. C.; ter Haar, E.; Binch, H. M.; Kay, D. P.; Patel, S. R.; Li, P. *J. Med. Chem.* **2005**, *48*, 1278.

(7) Alvarez, S. *Dalton Trans.* **2013**, *42*, 8617.

(8) Chein, R. J.; Corey, E. J. *Org. Lett.* **2010**, *12*, 132.

(9) (a) Groom, C. R.; Allen, F. H. *Angew. Chem., Int. Ed. Engl.* **2014**, *53*, 662. (b) Allen, F. H. *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *58*, 380. (c) Brameld, K. A.; Kuhn, B.; Reuter, D. C.; Stahl, M. *J. Chem. Inf. Model.* **2008**, *48*, 1. (d) Burling, F. T.; Goldstein, B. M. *Acta Crystallogr., Sect. B: Struct. Sci.* **1993**, *49*, 738.

(10) (a) Senger, S.; Convery, M. A.; Chan, C. E.; Watson, N. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5731. (b) Iwaoka, M.; Takemoto, S.; Tomoda, S. *J. Am. Chem. Soc.* **2002**, *124*, 10613. (c) Angyan, J. G.; Poirier, R. A.; Kucsman, A.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1987**, *109*, 2237. (d) Burling, F. T.; Goldstein, B. M. *J. Am. Chem. Soc.* **1992**, *114*, 2313. (e) Nagao, Y.; Hirata, T.; Goto, S.; Sano, S.; Kakehi, A.; Iizuka, K.; Shiro, M. *J. Am. Chem. Soc.* **1998**, *120*, 3104.

(11) Ames, R. S.; Lee, D.; Foley, J. J.; Jurewicz, A. J.; Tornetta, M. A.; Bautsch, W.; Settmacher, B.; Klos, A.; Erhard, K. F.; Cousins, R. D.; et al. *J. Immunol.* **2001**, *166*, 6341.

(12) (a) Chu, D. C. K.; Cho, J. H.; Kim, H.-J.; WO 2007047793A2, University of Georgia Research Foundation, Inc., USA, 2007. (b) Searls, T.; McLaughlin, L. W. *Tetrahedron* **1999**, *55*, 11985.