Bioorganic & Medicinal Chemistry Letters 21 (2011) 4545–4549

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Benzisothiazolones as modulators of macrophage migration inhibitory factor

William L. Jorgensen [a,*], Alexander Trofimov [a], Xin Du [b], Alissa A. Hare [a], Lin Leng [b], Richard Bucala [b,*]

[a] Department of Chemistry, Yale University, New Haven, CT 06520-8107, USA
[b] Department of Medicine, Yale University School of Medicine, New Haven, CT 06520-8031, USA

ARTICLE INFO

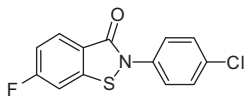
Article history:
Received 30 March 2011
Revised 27 May 2011
Accepted 31 May 2011
Available online 12 June 2011

Keywords:
MIF inhibitors
Benzisothiazolone
Cysteine modification
Macrophage migration inhibitory factor
MIF antagonists
Structure-based drug design

ABSTRACT

Substituted *N*-phenylbenzisothiazolones have been investigated as inhibitors of the tautomerase activity of the proinflammatory cytokine MIF (macrophage migration inhibitory factor). Numerous compounds were found to possess antagonist activity in the low micromolar range with the most potent being the 6-hydroxy analog **1w**. Compound **1w** and the *p*-cyano analog **1c** were also shown to exhibit significant inhibition of the binding of MIF to its transmembrane receptor CD74. Consistently, both compounds were also found to retard the MIF-dependent phosphorylation of ERK1/2 in human synovial fibroblasts.

The cytokine MIF (macrophage migration inhibitory factor) is emerging as an important therapeutic target for inflammatory, autoimmune, and hyperproliferative diseases.[1] MIF possesses multiple biological functions and it participates in many cellular processes. In addition to activating mitogen-activated protein kinases (MAPK),[2] MIF directly down-regulates p53 expression and function,[3] and it counter-regulates the expression of glucocorticoids.[4] Via binding to its cellular receptor CD74, MIF can promote cell survival through activation of the PI3K/Akt pathway. The roles of MIF in cardiovascular diseases, inflammation, and innate immune responses are well documented.[1,5,6] MIF is implicated in multiple disorders including rheumatoid arthritis,[7] glomerulonephritis,[8] diabetes,[9] atherosclerosis,[10] sepsis,[11] asthma,[12] and acute respiratory distress syndrome.[13] Moreover, it has been shown that inhibition of MIF or CD74 attenuates prostate cancer cell growth.[14] In addition to its activities as a cytokine, MIF is an enzyme that catalyzes keto–enol tautomerization. It appears that the tautomerase active site is located in proximity to the MIF-CD74 binding site.[15] Thus, although evidence has not emerged that the enzymatic activity of MIF is significant in mammals, substantial effort has been directed at the discovery of small-molecule inhibitors of both MIF's enzymatic and signaling activities.[16] Our joint efforts began with virtual screening that yielded 11 structurally diverse inhibitors of MIF-CD74 binding with micromolar activities.[17] One series, with a benzoxazolone core, was further pursued and yielded antagonists with IC_{50} values as low as 7.5 nM in the tautomerase assay and 80 nM in the receptor binding assay.[18] In parallel de novo design efforts, triazole derivatives emerged as viable alternatives. This led to the report of additional antagonists, and to the first agonists of MIF-CD74 signaling.[19] Another promising hit from the virtual screening was 2-(4-chlorophenyl)-6-fluoro-benzo[*d*]isothiazol-3(2*H*)-one **1a** with an IC_{50} of 4.2 μM in the tautomerase assay.[17] Results of further explorations of benzisothiazolones are reported here.



1a

Our computational studies indicated that **1a** should bind to MIF in the manner illustrated in Figure 1. Notably, results of Monte Carlo free-energy perturbation calculations[20] found that (a) the orientation with the carbonyl group of **1a** pointing towards Ile64 is preferred by ca. 6 kcal/mol over the 180°-rotated alternative with the carbonyl group pointed towards Tyr95, and (b) substitutions at C5 or C6 with F, Cl, OH, OCH_3, and CN should be comparably viable and preferred over CH_3. This model received subsequent support from crystallographic studies of complexes of **2** and **3** with MIF.[21] Compounds **1a**, **2**, and **3** align well with the bicyclic ring systems inserted into the active site and with the fluorine of **1a** and bicyclic OH groups of **2** and **3** proximal to Asn97, and the C=O

* Corresponding authors.
E-mail addresses: william.jorgensen@yale.edu (W.L. Jorgensen), richard.bucala@yale.edu (R. Bucala).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.05.127

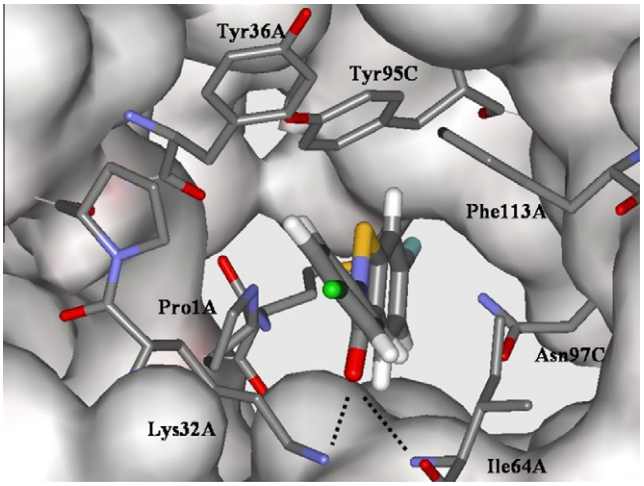
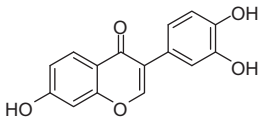
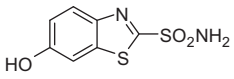


Figure 1. Computed structure of the complex of **1a** bound in the MIF tautomerase site after energy-minimization with MCPRO[20] starting with protein coordinates from the 1GCZ crystal structure.[16a]

groups of **1a** and **2** hydrogen-bonded to the NH of Ile64. The synthetic efforts were focused accordingly with particular interest in preparing the 6-OH analog in view of precedents indicating potential value of hydrogen-bonding with Asn97.[17] The structures of the synthesized analogs, **1b**–**1y**, are recorded in Table 1.



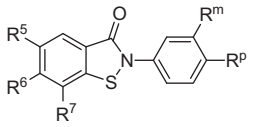
2



3

Synthetic routes used for the preparation of *N*-arylbenzisothiazolones **1b**–**1v** are summarized in Scheme 1. Compounds **1b**–**1l** were prepared starting from the easily available secondary arylamides[22] **4** via method A (Scheme 1). *Ortho*-lithiation of **4** with *sec*-butyllithium in the presence of tetramethylethylenediamine (TMEDA),[23] followed by quenching of the dianion with dimethyl disulfide afforded the corresponding arylmethylthioethers **5** in good yields. Sulfides **5** were heated with $NaIO_4$ in methanol to yield the sulfoxides **6**, which were cyclized with thionyl chloride to afford the desired *N*-arylbenzisothiazolones **1b**–**1l** in moderate to good yields.[24] Benzisothiazolones **1m**–**1p** were prepared via method B in Scheme 1. Introduction of sulfide functionality into arylamide **7** en route to **9** required protection of the bisactivated *ortho*-site of arylamides **7** with a trimethylsilyl group.[25] Thus, **7** was subjected to standard directed lithiation conditions followed by trapping of the generated dianion by TMS chloride to provide amides **8**. Then **8** was subjected to the second *ortho*-lithiation, dimethyl disulfide quench, and desilylation with tetrabutylamonium fluoride (TBAF) to yield the sulfides **9**. These products were subsequently oxidized and cyclized as in method A to afford the desired **1m**–**1p**. Analogs **1q**–**1t**, which possess functional groups that are incompatible with *ortho*-lithiation methodology, were prepared starting from the corresponding 2-fluorobenzoic acids **10** via method C. Benzoyl chlorides, formed by refluxing **10** in thionyl chloride, were reacted with anilines to afford amides **11** in good to excellent yields. For **1t**, the cyano group was introduced via Pd-catalyzed cyanation of the aryl bromide.[26] Amides **11** were subjected to S_nAr reaction with sodium thiomethoxide in DMSO to afford thioethers **12** in good yields. Finally, **12** was cyclized to **1q**–**1t** upon treatment with sulfuryl chloride.[27] Benzisothiazolones **1u**–**1v** were prepared as depicted in method D starting from readily available 5-bromo-2-fluorobenzamide **13**.[28] Cu-catalyzed N-arylation of **13** with 1-choro-4-iodobenzene (**14**) allowed preparation of arylamides **15** en route to **1u** and **1v** via the protocol in method C. Finally, preparation of hydroxyl-substituted analogs **1w**–**1y** required use of protecting groups, as detailed in Scheme 2. Precursors **1f**, **1h**, and **17** were prepared by the methods in Scheme 1 and deprotected in a final step.

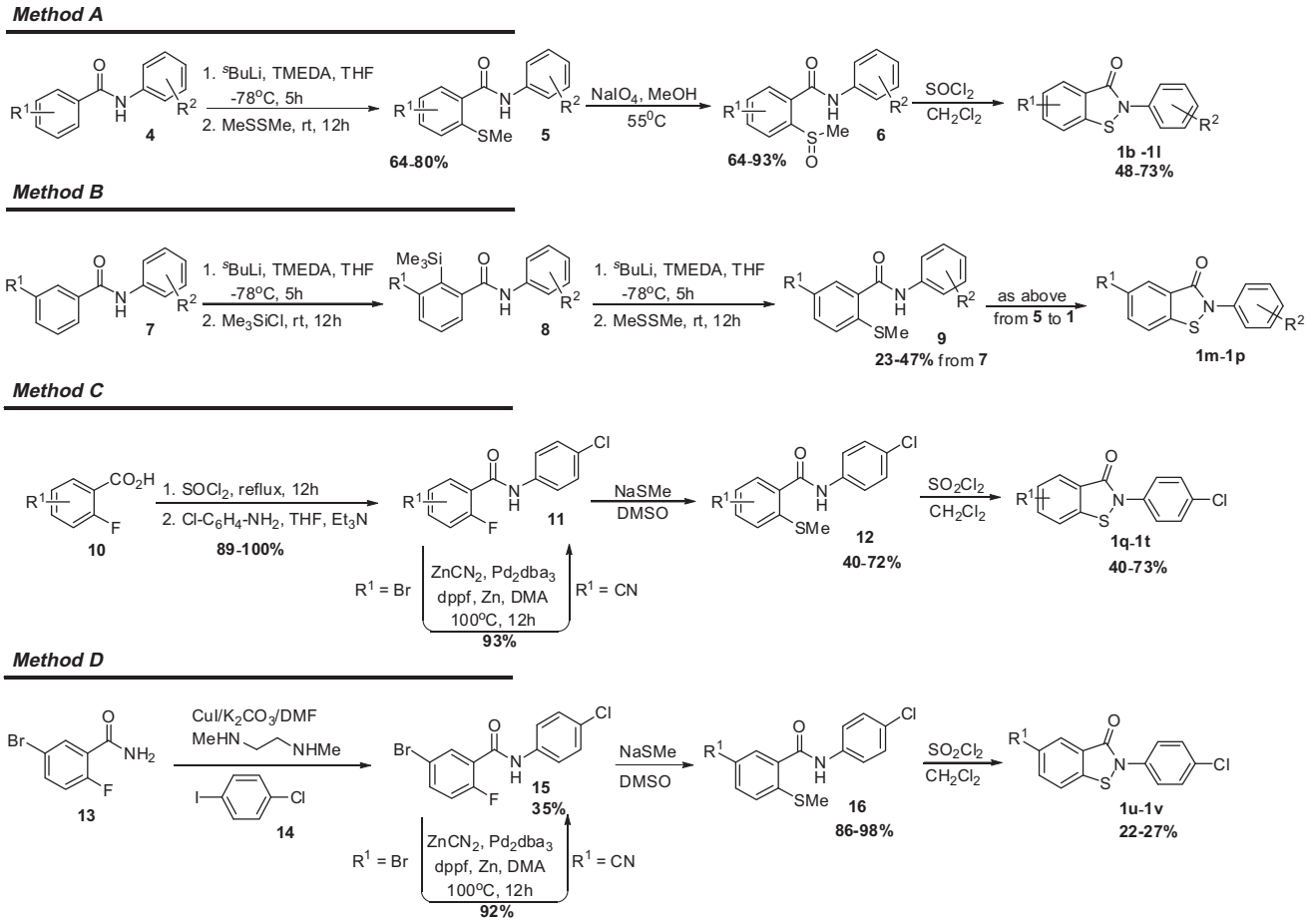
Table 1
Inhibitory activities for benzisothiazolones in the MIF tautomerase assay



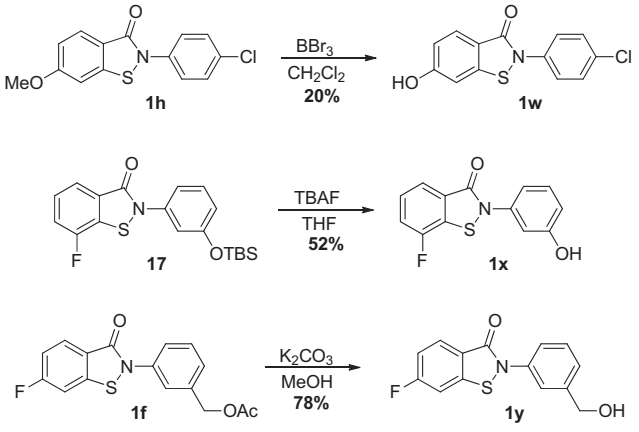
Compd	R^5	R^6	R^7	R^m	R^p	IC_{50} (μM)
1a	H	F	H	H	Cl	4.2
1b	H	F	H	H	OMe	6.2
1c	H	F	H	H	CN	6.4
1d	H	F	H	OMe	H	25
1e	H	F	H	Cl	H	5.0
1f	H	F	H	CH_2OAc	H	2.3
1g	H	F	H	CH_2OMe	H	2.4
1h	H	OMe	H	H	Cl	1.9
1i	H	H	F	OMe	H	8.0
1j	H	H	F	H	Cl	2.8
1k	H	H	Cl	H	Cl	6.6
1l	H	F	F	H	Cl	5.6
1m	F	H	H	H	Cl	5.6
1n	F	H	H	H	OMe	6.5
1o	F	F	H	H	Cl	4.9
1p	F	H	H	OMe	H	2.8
1q	NO_2	H	H	H	Cl	6.2
1r	CF_3	H	H	H	Cl	8.5
1s	H	Br	H	H	Cl	11
1t	H	CN	H	H	Cl	3.1
1u	Br	H	H	H	Cl	7.9
1v	CN	H	H	H	Cl	19
1w	H	OH	H	H	Cl	1.0
1x	H	H	F	OH	H	5.9
1y	H	F	H	CH_2OH	H	4.8

The ability of the *N*-arylbenzisothiazolones **1** to inhibit the tautomerase activity of MIF was evaluated with 4-hydroxyphenylpyruvate (4-HPP) as the substrate, as previously presented.[17,29] Human MIF, prepared recombinantly, was used throughout. All compounds show good activities ranging from 1 to 25 μM (Table 1). As expected from the structural model, there was little effect on the activities from varying the solvent-exposed substituents at the *para* (R^p) position (**1a**, **1b**, **1c**). Some substitutions at the *meta* position provided improvement, for example, **1f** and **1g**, while variation at the 6-position did lead to the most potent compounds, **1h** and **1w**. Indeed, the hydroxyl analog **1w** is the most active at 1 μM. We have found few compounds more active than this in the 4-HPP tautomerase assay including the covalent inhibitor 4-IPP (4-iodo-6-phenylpyrimidine), which yields an IC_{50} of 4.5 μM.[17–19]

Many of the compounds were also tested in the MIF-CD74 binding assay. As previously detailed, this assay features biotinylated MIF and immobilized CD74 ectodomain ($CD74^{73–232}$) with streptavidin conjugated alkaline phosphatase processing *p*-nitrophenylphosphate as the reporter.[17,30] Most of the present benzisothiazolones showed weak inhibitory activity that did not reach the 50% level in the micromolar range. The two strongest antagonists are **1c** and **1w**, which showed 49% inhibition of binding at 78 nM and 23% inhibition at 4 nM, respectively. A few of the compounds emerged as agonists[19]; the strongest is **1i**, which enhances the MIF-CD74 binding by 2.7-fold at 4 μM.



Scheme 1. Synthesis of *N*-arylbenzisothiazolones **1b**–**1v**.



Scheme 2. Final deprotection step in the syntheses of **1w**–**1y**.

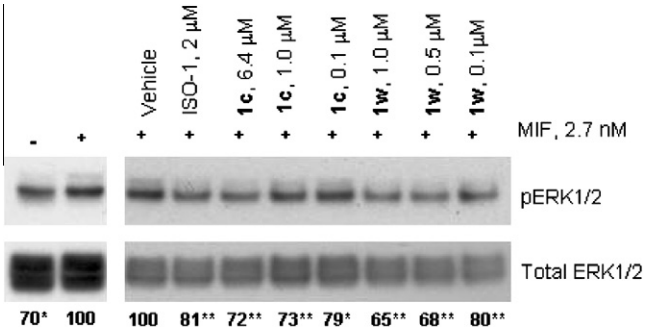
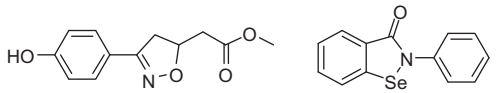


Figure 2. Relative potency of MIF antagonists in reducing MIF-dependent ERK1/2 phosphorylation in human synovial fibroblasts. The bottom numerals show the ratio of phosphorylated to total ERK protein relative to MIF alone from densitometric scanning. Values are expressed in relative units from two experiments. $^{*}P$ <0.05, $^{**}P$ <0.01 by *t*-test.

In view of these results, inhibition of MIF signal transduction by **1c** and **1w** was further investigated in human target cells, specifically, primary synovial fibroblasts. The expectation is that MIF-CD74 inhibition should reduce ERK (extracellular-signal-regulated kinase) phosphorylation, which is relevant to the control of hyperproliferative and autoimmune diseases.[31] Thus, the test compounds were added together with MIF for 30 min to 1×10^6 synovial fibroblasts cultured in DMEM/0.1% fetal bovine serum. The cells then were lysed and the intracellular contents of phospho-ERK1/2 and total ERK1/2 were quantified by specific antibodies and western blotting. The results in Figure 2 show that addition of MIF enhances ERK phosphorylation, while co-administration with **1c** and **1w** results in substantial reduction of phosphorylation. The most profound effects are obtained with the 6-hydoxy analog **1w**, which reduces the phosphorylation by 35% at 1 μM and still by 20% at 100 nM. For comparison, results are included for the well-studied MIF tautomerase inhibitor ISO-1[32]; the racemate yields a 19% reduction of ERK phosphorylation at 2 μM.



A final issue that was addressed was the possible covalent modification of MIF by the benzisothiazolones. Many MIF inhibitors have been reported to function in this manner by bonding to the nucleophilic Pro1 or Cys residues.[15b,33] Indeed, Ouertatani-Sakouhi et al. recently reported 12 covalent modifiers of Pro1 from high-throughput screening and mass spectrometric analysis[33]; these compounds feature reactive carbonyl functionality that is normally avoided when seeking reversible inhibitors.[34] However, they also demonstrated that the anti-inflammatory agent ebselen inhibits MIF by covalent attachment specifically to Cys80 among the three Cys options (56, 59, and 80).[33] The modification causes disruption of MIF trimer assembly and, therefore, negates formation of the tautomerase active sites.[33] Besides the structural similarity of **1a** and ebselen, it has also been shown that some *N*-arylbenzisothiazolones can react with cysteines yielding opening of the benzisothiazolone ring and disulfide formation.[35] The latter process should be facilitated by electron-withdrawing substituents in the *para* position of the N-phenyl ring as in the reported case with a *p*-sulfamoyl group.

Thus, mass spectrometric studies were pursued to assess possible covalent attachment of **1a** to MIF in a similar manner to the prior investigation of 4-IPP and a benzoxazolone MIF inhibitor.[18] Equimolar quantities of human MIF and **1a** were mixed (30 μM of each reactant in 0.01 mL) for 1 h at 25 °C and the reaction mixture was analyzed (Bruker 9.4T FT-ICR). A small peak appeared at the mass position expected for the covalent attachment of one molecule of **1a** with a MIF monomer (12,616 Da), as shown in Figure 3. Analysis of the four highest peaks in the MIF alone and MIF + **1a** isotopic distributions indicates that ca. 3% of the MIF is modified by **1a**. With 4-IPP, which has a similar IC_{50} (4.5 μM) in the tautomerase assay, ca. 20% of the protein was modified.[18] Further FT-ICR MS analysis of a trypsin-digested sample revealed a peptide of M = 6083 Da that corresponds to the covalent adduct of the MIF^{12-66} fragment and **1a** (Fig. 3). These data are consistent with covalent attachment of MIF and **1a** at Cys56 or Cys59. The fact that Cys59 is well-buried in the structure of the MIF trimer shifts the likelihood of the modified residue towards being Cys56. While the catalytic Pro1 is not modified by **1a**, cysteine modification could influence MIF conformational integrity and account for alterations in tautomerization

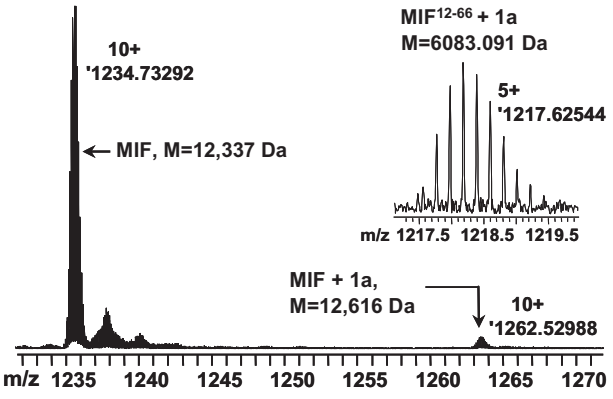


Figure 3. Mass spectrometric results demonstrating minor covalent modification of MIF by **1a**. The inset shows the product after trypsin digestion of the modified protein.

activity and receptor binding functions. However, the extent of covalent modification appears small, and it is unlikely that it can account for the absolute activities in Table 1 or of the relative values. For example, electron-withdrawing groups should make the benzisothiazolones more reactive electrophiles; however, this is not apparent in comparisons of the IC_{50} values such as for **1f** and **1g** versus **1a** and **1c**, and in comparing the isomeric **1t** and **1v**, where **1v** would be expected to be more reactive towards nucleophilic attack at the conjugating *para*-sulfur site.

In summary, *N*-phenylbenzisothiazolones have been examined for their ability to inhibit the tautomerase and signaling activities of the cytokine MIF. Numerous analogs have been reported with activities in the low micromolar range for inhibition of the tautomerase activity. Though the expectation is that they are reversible inhibitors that bind near the tautomerase active site, mass spectroscopic investigation of the original virtual screening hit **1a** demonstrated that it yields a small amount of covalent modification of Cys56 or Cys59 of MIF. The most potent MIF tautomerase inhibitor reported here is the 6-hydroxy analog **1w** at 1 μM. This compound was also shown to cause striking retardation of ERK phosphorylation in synovial fibroblasts. Investigations continue in several veins including evaluation of the anti-inflammatory and anti-proliferative activities of the compounds.

Acknowledgments

Gratitude is expressed to T. Lam and E. Voss of the Keck Biotechnology Facility for the mass spectrometric analyses, and to the National Institutes of Health (AI042310, AR049610, AR050498, GM032136) and the Treat B. Johnson Fund at Yale for support.

References and notes

1. (a) Lolis, E.; Bucala, R. *Expert Opin. Ther. Targets* **2003**, *7*, 153; (b) Greven, D.; Leng, L.; Bucala, R. *Expert Opin. Ther. Targets* **2010**, *14*, 253.
2. (a) Santos, L. L.; Lacey, D.; Yang, Y.; Leech, M.; Morand, E. F. *J. Rheumatol.* **2004**, *31*, 1038; (b) Mitchell, R. A.; Metz, C. N.; Peng, T.; Bucala, R. *J. Biol. Chem.* **1999**, *274*, 18100.
3. Mitchell, R. A.; Liao, H.; Chesney, J.; Fingerle-Rowson, G.; Baugh, J.; David, J.; Bucala, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 345.
4. (a) Calandra, T.; Bernhagen, J.; Metz, C. N.; Spiegel, L. A.; Bacher, M.; Donnelly, T.; Cerami, A.; Bucala, R. *Nature* **1995**, *377*, 68; (b) Leech, M.; Metz, C.; Bucala, R.; Morand, E. F. *Arthritis Rheum.* **2000**, *43*, 827.
5. (a) Zernecke, A.; Bernhagen, J.; Weber, C. *Circulation* **2008**, *117*, 1594; (b) Herder, C.; Illig, T.; Baumert, J.; Mueller, M.; Klopp, N.; Khuseyinova, N.; Meisinger, C.; Martin, S.; Thorand, B.; Koenig, W. *Atherosclerosis* **2008**, *200*, 380.
6. Calandra, T.; Roger, T. *Nat. Rev. Immunol.* **2003**, *3*, 791.
7. Kim, H.-R.; Park, M.-K.; Cho, M.-L.; Yoon, C.-H.; Lee, S.-H.; Park, S.-H.; Leng, L.; Bucala, R.; Kang, I.; Choe, J.; Kim, H.-Y. *J. Rheumatol.* **2007**, *34*, 927.
8. Lan, H. Y. *Nephron* **2008**, *109*, 79.
9. Sanchez-Zamora, Y.; Terrazas, L. I.; Vilches-Flores, A.; Leal, E.; Juarez, I.; Whitacre, C.; Kithcart, A.; Pruitt, J.; Sielecki, T.; Satoskar, A. R.; Rodriguez-Sosa, M. *FASEB J.* **2010**, *24*, 2583.
10. Noels, H.; Bernhagen, J.; Weber, C. *Trends Cardiovascul. Med.* **2009**, *19*, 76.
11. Bozza, M.; Satoskar, A. R.; Lin, G.; Lu, B.; Humbles, A. A.; Gerard, C.; David, J. R. *J. Exp. Med.* **1999**, *189*, 341.
12. Yamaguchi, E.; Nishihira, J.; Shimizu, T.; Takahashi, T.; Kitashiro, N.; Hizawa, N.; Kamishima, K.; Kawakami, Y. *Clin. Exp. Allergy* **2000**, *30*, 1244.
13. Lai, K. N.; Leung, J. C. K.; Metz, C. N.; Lai, F. M.; Bucala, R.; Lan, H. Y. *J. Pathol.* **2003**, *199*, 496.
14. Meyer-Siegler, K. L.; Iczkowski, K. A.; Leng, L.; Bucala, R.; Vera, P. L. *J. Immunol.* **2006**, *177*, 8730.
15. (a) Senter, P. D.; Al-Abed, Y.; Metz, C. N.; Benigni, F.; Mitchell, R. A.; Chesney, J.; Han, J.; Gartner, C. G.; Nelson, S. D.; Todar, G. J.; Bucala, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 144; (b) Winner, M.; Meier, J.; Zierow, S.; Rendon, B. E.; Crichlow, G. V.; Riggs, R.; Bucala, R.; Leng, L.; Smith, N.; Lolis, E.; Trent, J. O.; Mitchell, R. A. *Cancer Res.* **2008**, *68*, 7253; (c) McLean, L. R.; Zhang, Y.; Li, H.; Choi, Y.-M.; Han, Z.; Li, Y. *Bioorg. Med. Chem. Lett.* **2009**, *20*, 1821.
16. For reviews, see: (a) Orita, M.; Yamamoto, S.; Katayama, N.; Fujita, S. *Curr. Pharm. Des.* **2002**, *8*, 1297; (b) Garai, J.; Lorand, T. *Curr. Med. Chem.* **2009**, *16*, 1091.
17. Cournia, Z.; Leng, L.; Gandavadi, S.; Du, X.; Bucala, R.; Jorgensen, W. L. *J. Med. Chem.* **2009**, *52*, 416.
18. Hare, A. A.; Leng, L.; Gandavadi, S.; Du, X.; Cournia, Z.; Bucala, R.; Jorgensen, W. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5811.

19. Jorgensen, W. L.; Gandavadi, S.; Du, X.; Hare, A. A.; Trofimov, A.; Leng, L.; Bucala, R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7033.
20. Jorgensen, W. L.; Tirado-Rives, J. *J. Comput. Chem.* **2005**, *26*, 1689.
21. McClean, L. R.; Zhang, Y.; Li, H.; Choi, Y.-M.; Han, Z.; Vaz, R. J.; Li, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1821.
22. Shaw, A. Y.; Chen, Y.-R.; Tsai, C.-H. *Synth. Comm.* **2009**, *39*, 2647.
23. Metallinos, C.; Nerdinger, S.; Snieckus, V. *Org. Lett.* **1999**, *1*, 1183.
24. Uchida, Y.; Kozuka, S. *Chem. Comm.* **1981**, *11*, 510.
25. Mills, R. J.; Taylor, N. J.; Snieckus, V. *J. Org. Chem.* **1989**, *54*, 4372.
26. Didier, D.; Sergeyev, S. *Tetrahedron* **2007**, *63*, 3864.
27. Zlotin, S. G.; Kislitsin, P. G.; Podgursky, A. I.; Samet, A. V.; Semenov, V. V.; Buchanan, A. C., III; Gakh, A. A. *J. Org. Chem.* **2000**, *65*, 8439.
28. Perez-Medrano, A.; Donnelly-Roberts, D. L.; Honore, P.; Hsieh, G. C.; Namovic, M. T.; Peddi, S.; Shuai, Q.; Wang, Y.; Faltynek, C. R.; Jarvis, M. F.; Carroll, W. A. *J. Med. Chem.* **2009**, *52*, 3366.
29. Bernhagen, J.; Mitchell, R. A.; Calandra, T.; Voelter, W.; Cerami, A.; Bucala, R. *Biochemistry* **1994**, *33*, 14144.
30. Shi, X.; Leng, L.; Wang, T.; Wang, W.; Du, X.; Li, J.; McDonald, C.; Chen, Z.; Murphy, J. W.; Lolis, E.; Noble, P.; Knudson, W.; Bucala, R. *Immunity* **2006**, *25*, 595.
31. (a) Bucala, R.; Lolis, E. *Drug News Perspect.* **2005**, *18*, 417; (b) Ohori, M. *Drug News Perspect.* **2008**, *21*, 245.
32. Lubetsky, J. B.; Dios, A.; Han, J.; Aljabari, B.; Ruzsicska, B.; Mitchell, R.; Lolis, E.; Al Abed, Y. *J. Biol. Chem.* **2002**, *277*, 24976.
33. Ouertatani-Sakouhi, H.; El-Turk, F.; Fauvet, B.; Cho, M.-K.; Karpinar, D. P.; Le Roy, D.; Dewor, M.; Roger, T.; Bernhagen, J.; Calandra, T.; Zweckstetter, M.; Lashuel, H. A. *J. Biol. Chem.* **2010**, *285*, 26581.
34. Rishton, G. M. *Drug Discovery Today* **2003**, *8*, 86.
35. Sanchez, J. P. *J. Heterocycl. Chem.* **1997**, *34*, 1463.