Bioorganic & Medicinal Chemistry Letters 18 (2008) 6142–6146

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Pyrrolo-pyrimidones: A novel class of MK2 inhibitors with potent cellular activity

Achim Schlapbach *, Roland Feifel, Stuart Hawtin, Richard Heng, Guido Koch, Henrik Moebitz, Laszlo Revesz, Clemens Scheufler, Juraj Velcicky, Rudolf Waelchli, Christine Huppertz

Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 11 September 2008
Revised 1 October 2008
Accepted 2 October 2008
Available online 11 October 2008

Keywords:
MK2
MAPKAPK-2
TNFα inhibition
Inflammation
Rheumatoid arthritis

ABSTRACT

Pyrrolo-pyrimidones of the general structure **1** were synthesized and evaluated for their potential as MK2 inhibitors. Potent derivatives were discovered which inhibit MK2 in the nanomolar range and show potent inhibition of cytokine release from LPS-stimulated monocytes. These derivatives were shown to inhibit phosphorylation of hsp27, a downstream target of MK2 and are modestly selective in a panel of 28 kinases.

© 2008 Elsevier Ltd. All rights reserved.

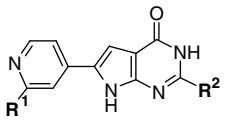
The first anti-TNFα (tumor necrosis factor alpha) biologicals were introduced into clinical practice in the late 1990s. Since then they became very successful in the treatment of several autoimmune diseases like rheumatoid arthritis (RA), Crohn's disease or psoriasis.[1] Anti-TNF therapeutics meanwhile became the 'gold-standard' in RA therapy, which fostered the search for new anti-cytokine targets which are amenable for modulation using low-molecular weight compounds. Considerable efforts have focused on the discovery and development of p38 inhibitors,[2] yet, the first compound of this class has still not reached the market. While p38 is still an attractive target, the search for new, potentially more selective components in the p38 signaling cascade is ongoing.[3] Such a target could be mitogen-activated protein kinase activated protein kinase 2 (MAPKAPK2 or MK2), a serine/threonine kinase downstream of p38 which has been shown to play a critical role in TNFα signaling, both in vitro and in vivo.[4] We now disclose our efforts to identify and optimize low-molecular weight inhibitors of MK2, their structure–activity relationships and cellular profile.

Recently, several reports of MK2 inhibitors from a variety of structural classes were published, amongst them aminocyanopyridines[5], tetrahydro-carbolines[6], indole derivatives,[7] and pyrrolo-pyridones.[8] In this communication we will present our results on a related compound class, namely pyrrolo-pyrimidones, exemplified by the general structure **1** (Fig. 1)

In a first effort to optimize this series we evaluated position R^1 utilizing aryl, heteroaryl and styrene substituents. For substituent R^2 small alkyl, benzyl and amino groups were explored (Table 1). Whereas aryl substituents in position R^1 yielded compounds which inhibit MK2[9] in the micromolar range, potency improved significantly when the more flexible phenylethenyl (styryl) substituents were introduced. In particular, fluorostyryl derivative **9** potently inhibited MK2 with an IC_{50} value of 0.20 μM and more importantly displayed cellular activity, that is, inhibition of TNFα release from LPS-stimulated hPBMCs[10] in the same activity range.

Variation of R^2 did not yield any major improvements. Small substituents like an amino or methyl group are tolerated. Larger alkyl groups (CF3, *n*-butyl) reduce potency, but interestingly further increase in size as in the case of the benzyl-derivative **14**, generated a more potent inhibitor of MK2, albeit without activity in our cellular assay.

To further explore the styrene motif and to improve physicochemical properties, for example, solubility, a series of substituted styrene derivatives were synthesized and evaluated (Table 2). The



1

Figure 1. Pyrrolo-pyrimidone core structure.

* Corresponding author. Tel.: +41 61 324 4308; fax: +41 61 324 8847.
E-mail address: achim.schlapbach@novartis.com (A. Schlapbach).

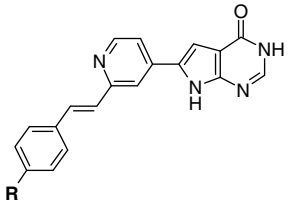
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.039

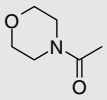
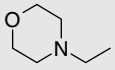
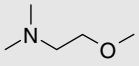
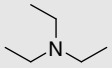
Table 1
In vitro activity of pyrrolo-pyrimidones

Compound	R^1	R^2	MK2[a] IC_{50} [μM]	hPBMC[b] IC_{50} [μM]
2	4-Fluorophenyl	H	1.30	n.d.
3	3-Fluorophenyl	H	1.23	n.d.
4	4-Methoxyphenyl	H	0.55	>10
5	3-Methoxyphenyl	H	5.20	n.d.
6	4-Morpholino-methylphenyl	H	1.45	n.d.
7	4-Cyclopentyl-benzamido	H	0.19	10
8	Phenylethenyl	H	0.17	2.30
9	*p*-Fluorostyryl	H	0.20	0.35
10	Phenylethenyl	NH_2	0.29	1.50
11	Phenylethenyl	CF_3	1.70	n.d.
12	Phenylethenyl	CH_3	0.35	1.10
13	Phenylethenyl	n-C_4H_9	1.26	n.d.
14	Phenylethenyl	Benzyl	0.34	>10

IC_{50} values are reported as the mean of ⩾2 experiments.
[a] MK2 enzyme assay is performed as described.[9]
[b] Inhibition of LPS stimulated release of TNFα from hPBMCs.[10]

Table 2
Effect of styrene substitution on in vitro activity of pyrrolo-pyrimidones



Compound	R	MK2[a] IC_{50} [μM]	hPBMC[b] IC_{50} [μM]	Solubility[c] [g/l]
9	F-	0.20	0.35	0.003
15		0.042	0.15	0.009
16		0.051	0.11	0.041
17		0.082	0.04	0.41
18		0.150	0.38	n.d.

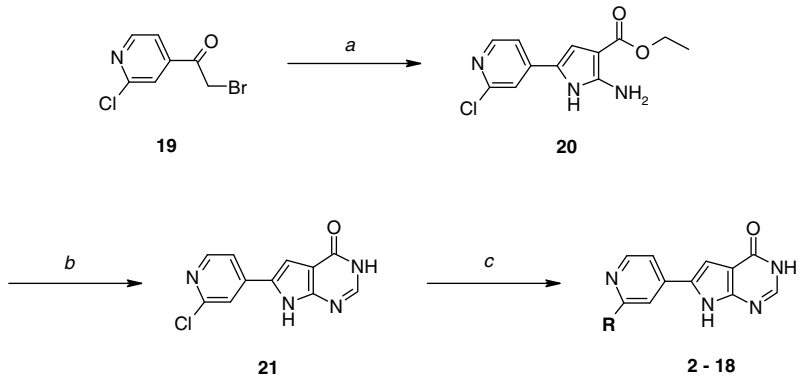
IC_{50} values are reported as the mean of ⩾2 experiments.
[a] MK2 enzyme assay is performed as described.[9]
[b] Inhibition of LPS stimulated release of TNFα from hPBMCs.[10]
[c] Thermodynamic solubility at pH 6.8.

addition of substituents to the styrene para position did not only influence solubility but also improved potency up to ca. 5-fold. Compounds **15–17** now display high MK2 activity (40–80 nM) and show strong inhibition of TNFα release from LPS-stimulated hPBMCs.

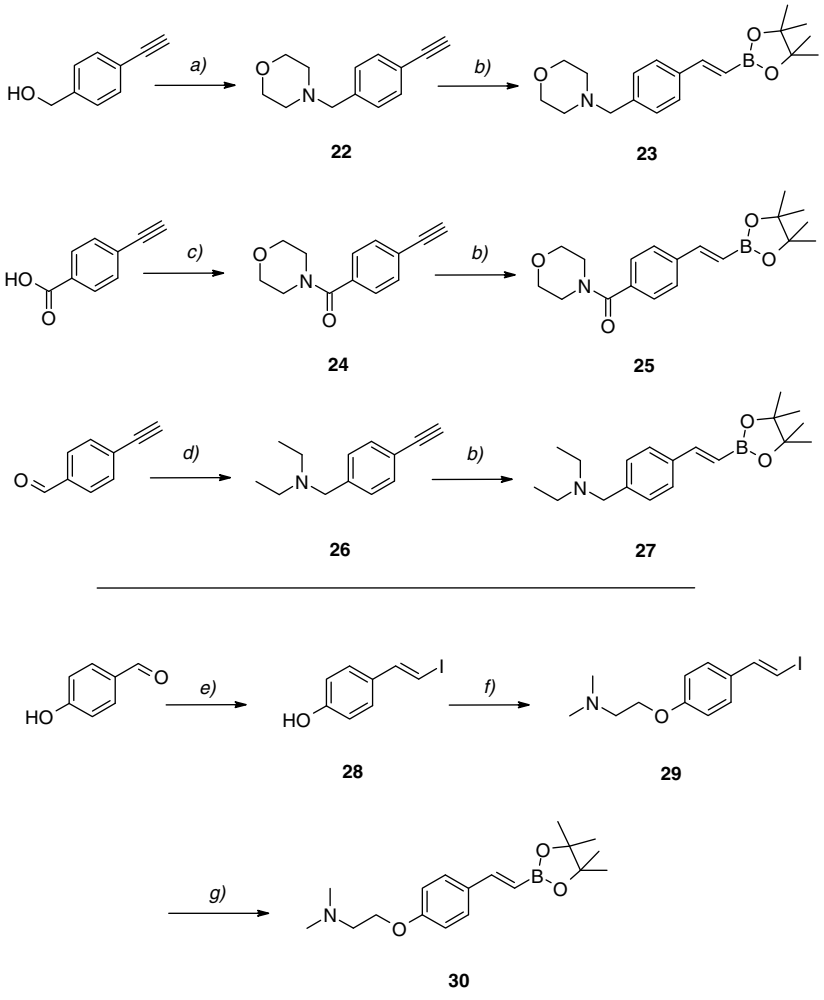
The synthesis of pyrrolo-pyrimidones started with bromo-ketone **19**. Cyclo-condensation with ethyl 3,3-diamino-acrylate[11] in methanol yielded the desired amino-pyrrole **20**, which when treated with formamidine under forcing conditions provided the pyrrolo-pyrimidone core structure **21**. The pyridyl-2-substituent was finally introduced by Suzuki-coupling with the corresponding boronates (Scheme 1).

Boronates which were not commercially available were synthesized either by hydroboration of the corresponding alkynes, or by a Takai-olefination[12]/metalation sequence (Scheme 2). Thus, amination of ethynylbenzyl alcohol with morpholine under Zaragoza conditions[13] yielded **22**, which in turn could be hydroborated smoothly with pinacolborane using Wilkinson's catalyst to give the boronate **23**. Compounds **25** and **27** were synthesized in the same manner. Alternatively *p*-hydroxybenzaldehyde was treated with iodoform and chromium chloride to give vinyl iodide **28**. The desired boronate **30** was then obtained after alkylation of the phenol, iodine lithium exchange and transmetalation with 2-isopropoxy-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane.

To further explore features which may be necessary for potent MK2 inhibition, derivatives lacking either the pyrrole or the pyrimidine N-H were synthesized. *N*-Methyl derivative **31** was obtained simply by alkylation of **16** with methyliodide. The synthesis of **34**



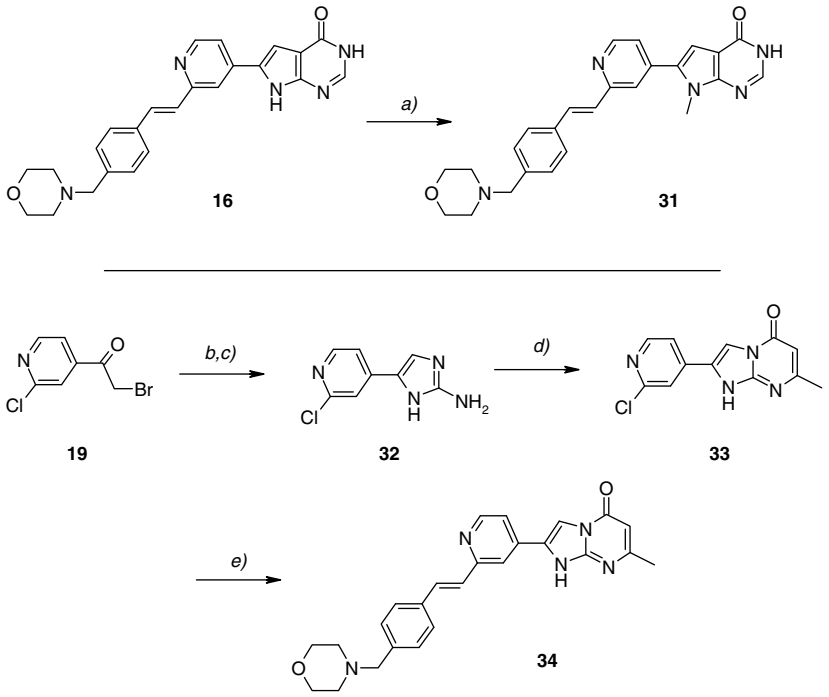
Scheme 1. Synthesis of pyrrolo-pyrimidone derivatives. Reagents and conditions: (a) ethyl 3,3-diamino-acrylate, $NaHCO_3$, methanol, rt 16 h; (b) formamidine, 1-butanol, reflux, 48 h; (c) boronate, $Pd(PPh_3)_2Cl_2$, 2 N $NaHCO_3$/1-propanol, reflux, 16 h (or microwave, 160 °C, 15 min), 10–75% (over three steps).



Scheme 2. Synthesis of styryl-boronates. Reagents and conditions: (a) Morpholine, cyanomethyl-trimethyl-phosphonium iodide, ethyl-diisopropyl-amine, propionitrile, reflux, 16 h, 95%; (b) 4,4,5,5-tetramethyl-[1,3,2]dioxaborolane, $Rh(PPh_3)_3Cl$, dichloromethane, rt, 24 h, 65%; (c) morpholine, EDC, dichloromethane, rt, 24 h, 73%; (d) diethylamine, $NaCNBH_3$, methanol/acetic acid, 93:7, rt, 16 h, 62%; (e) CHI_3, $CrCl_2$, 0 °C, 1 h, 73%; (f) dimethylaminoethyl chloride, K_2CO_3, acetone, 50 °C, 16 h, 68%; (g) 2-isopropoxy-4,4,5,5-tetramethyl-[1,3,2]dioxaboro lane, *t*-BuLi, THF, −78 °C, 1 h; then rt, 2 h, 60%.

proceeded via the amino-imidazole **32**, which was condensed with ethyl acetoacetate to provide imidazolo-pyrimidone **33**. Finally, Suzuki-coupling with boronate **23** led to compound **34** (Scheme 3). Compounds **31** and **34** were both significantly less potent MK2 inhibitors, with IC_{50} values of ca. 1.3 μM. This demonstrates the importance of both H-donors for potent MK2 inhibition.

Styrene derivatives **16** and **17** which were found to be both potent MK2 inhibitors and active in the cellular assay were further evaluated for their selectivity towards other kinases. Testing in a panel of 28 kinases revealed a modest degree of selectivity (Table 3). Kinases which showed strongest inhibition were the closely related kinase MK5 in addition to Aurora A, GSK3β, Jak3, JNK2, Kdr,



Scheme 3. Synthesis of **31** and **34**. Reagents and conditions: (a) NaH, MeI, DMF, rt, 16 h, 15%; (b) acetyl guanidine, acetonitrile reflux, 16 h, 50%; (c) cat H_2SO_4, MeOH/water, reflux, 16 h, 45%; (d) ethyl acetoacetate, ammonium acetate, neat 140 °C; (e) **23**, $Pd(PPh_3)_2Cl_2$, 2 N $NaHCO_3$/1-propanol, microwave, 160 °C, 15 min, 17% over two steps.

and c-met. Kinases which would be predicted to interfere with our cellular readout (TNFα release) were not (p38) or only moderately (JNK) inhibited. Furthermore, both compounds showed no major effect on cytotoxicity as assessed on the proliferation of THP-1 cells and mouse bone marrow derived cells.

It was important to demonstrate that the inhibition of TNFα release from PBMCs seen with **16** and **17** directly results from inhibition of MK2. Thus, we determined if the phosphorylation of hsp27, a direct downstream target of MK2[14] was also inhibited. Phosphorylated hsp27 is hardly detectable under basal conditions, and strongly induced upon stimulation with LPS (Fig. 2). Compounds **16** and **17** inhibited the LPS-stimulated phosphorylation of hsp27 dose dependently, while total hsp27 was not affected.[15] This suggests that perturbation of cytokine release is indeed mediated via inhibition of MK2.[16]

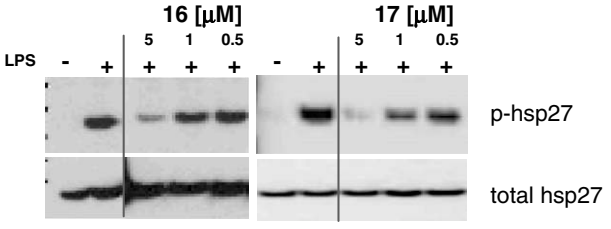


Figure 2. Inhibition of hsp27 phosphorylation in LPS-stimulated THP-1 cells.[15]

Table 3
Kinase selectivity of compounds **16** and **17**

Kinase	Compound **16** IC_{50} [μM]	Compound **17** IC_{50} [μM]
Alk	>10	4.1
Aurora A	0.093	0.28
Btk	>10	>10
CDK2	>10	8.2
ERK2	1.1	2.6
EphA4	>10	5.6
EphB4	1.7	1.4
FGFR-4	>10	6.8
GSK3b	0.30	0.53
HER1	>10	3.3
HER2	>10	9.5
IGF1R	>10	6.4
Ins1R	>10	7.8
Jak2	1.3	3.6
Jak3	0.31	1.0
JNK1	4.0	0.96
JNK2	0.95	0.24
Kdr	0.10	0.19
Lck	6.0	4.3
MK5	0.024	0.028
PDK1	2.2	3.4
PDGFRa	2.0	3.3
PKA	6.7	>10
PKB	>10	>10
Syk	6.2	2.5
c-Kit	5.2	6.1
c-Met	0.085	0.14
p38	>10	>10

In summary, we have discovered a new class of MK2 inhibitors: Pyrrolo-pyrimidones. Essential features for potent inhibition of MK2 within this compound class are a styrene substituent, optimally substituted with a polar head group as well as the pyrrole- and pyrimidone NH donors. Compounds **16** and **17** represent derivatives, which potently inhibit MK2 with modest selectivity in a panel of 28 kinases and exert cellular activity by inhibition of both TNF release from LPS-stimulated PBMCs and hsp27 phosphorylation.

Acknowledgments

We are very grateful to our colleagues from the NIBR kinase platform for providing in vitro kinase and selectivity data as well as the NIBR Prep Labs for the syntheses of valuable intermediates.

References and notes

1. Mousa, S. A.; Goncharuk, O.; Miller, D. *Expert Opin. Biol. Ther.* **2007**, *7*, 617.
2. Dominguez, C.; Powers, D. A.; Tamayo, N. *Curr. Opin. Drug Disc. Dev.* **2005**, *8*, 421; Schindler, J. F.; Monahan, J. B.; Smith, W. G. J. *Dent. Res.* **2007**, *86*, 800.
3. Zhang, J.; Shen, B.; Lin, A. *Trends Pharm. Sci.* **2007**, *28*, 286.
4. Kotlyarov, A.; Neininger, A.; Schubert, C.; Eckert, R.; Birchmeier, C.; Volk, H.-D.; Gaestel, M. *Nat. Cell. Biol.* **1999**, *1*, 94; Kotlyarov, A.; Yannoni, Y.; Fritz, S.; Laaß, K.; Telliez, J.-B.; Pitman, D.; Lin, L.-L.; Gaestel, M. *Mol. Cell. Biol.* **2002**, *22*, 4827; Neininger, A.; Kontoyiannis, D.; Kotlyarov, A.; Winzen, R.; Eckert, R.; Volk, H.-D.; Holtmann, H.; Kollias, G.; Gaestel, M. *J. Biol. Chem.* **2002**, *277*, 3065.
5. Anderson, D. R.; Hegde, S.; Reinhard, E.; Gomez, L.; Vernier, W. F.; Lee, L.; Liu, S.; Sambandam, A.; Snider, P. A.; Masih, L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1587.
6. Wu, J.-P.; Wang, J.; Abeywardane, A.; Andersen, D.; Emmanuel, M.; Gautschi, E.; Goldberg, D. R.; Kashem, M. A.; Lukas, S.; Mao, W.; Martin, L.; Morwick, T.; Moss, N.; Pargellis, C.; Patel, U. R.; Patnaude, L.; Peet, G. W.; Skow, D.; Snow, R. J.; Ward, Y.; Werneburg, B.; White, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4664; Trujillo, J. I.; Meyers, M. J.; Anderson, D. R.; Hegde, S.; Mahoney, M. W.; Vernier, W. F.; Buchler, I. P.; Wu, K. K.; Yang, S.; Hartmann, S. J.; Reitz, D. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4657.
7. Goldberg, D. R.; Choi, Y.; Cogan, D.; Corson, M.; DeLeon, R.; Gao, A.; Gruenbaum, L.; Hao, M. H.; Joseph, D.; Kashem, M. A.; Miller, C.; Moss, N.; Netherton, M. R.; Pargellis, C. P.; Pelletier, J.; Sellati, R.; Skow, D.; Torcellini, C.; Tseng, Y.-C.; Wang, J.; Wasti, R.; Werneburg, B.; Wu, J. P.; Xiong, Z. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 938; Xiong, Z.; Gao, D. A.; Cogan, D. A.; Goldberg, D. R.; Hao, M.-H.; Moss, N.; Pack, E.; Pargellis, C.; Skow, D.; Trieselmann, T.; Werneburg, B.; White, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1994.
8. Anderson, D. R.; Meyers, M. J.; Vernier, W. F.; Mahoney, M. W.; Kurumbail, R. G.; Caspers, N.; Poda, G. I.; Schindler, J. F.; Reitz, D. B.; Mourey, R. J. *J. Med. Chem.* **2007**, *50*, 2647.
9. MK2 was pre-activated in kinase buffer (25 mM Tris–HCl, pH 7.5, 25 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 25 mM $MgCl_2$, 20 μM DTT) containing 5 μM ATP, 150 μg/ml human MK2, 30 μg/ml active human p38α for 30 min at 22 °C. For MK2 inhibition, reactions contained compound (10 μl; 0.5% DMSO final) or vehicle control, 250 nM hsp27 peptide biotinyl-AYSRALSRQLSSGVSEI-R-COOH as substrate (10 μl) and activated MK2 mix (10 μl) containing ATP (5 μM final). Following incubation at 22 °C for 45 min, reactions were terminated with 125 μM EDTA (10 μl). Samples (10 μl) were transferred to black 384-well plates for detection of p-hsp27 by time-resolved fluorescence resonance energy transfer using an antibody mix (10 μl) containing a rabbit anti-phospho-hsp27 (Ser^{82}) antibody (2.5 nM, Upstate), and anti-rabbit europium-labeled secondary antibody LANCE Eu-W1024 (3.6 nM; Perkin Elmer) as fluorescence donor along with streptavidin SureLight-APC (6.25 nM; Perkin Elmer) as acceptor. Following incubation at 22 °C for 90 min, the FRET ratio 665/620 nm was determined. Individual IC_{50} values of compounds were determined by nonlinear regression after fitting of curves to the experimental data using Excel XL fit 4.0 (Microsoft).
10. Human peripheral blood mononuclear cells (hPBMCs) were prepared from the peripheral blood of healthy volunteers using Ficoll-Plaque Plus (Amersham) density separation. Cells were seeded at a 1×10^5 cells/well in 96-well plates in RPMI 1640 medium (Invitrogen) containing 10% (v/v) fetal calf serum. After pre-incubation with serial dilutions of test compound (0.25% v/v DMSO final) for 30 min at 37 °C, cells were stimulated with the addition of IFNγ (10 ng/ml) and lipopolysaccharide (LPS) (5 μg/ml) per well and incubated for 3 h at 37 °C. Following a brief centrifugation, supernatant (10 μl) samples from each well are measured against a TNFα calibration curve using a HTRF TNFα kit (CisBio).
11. Meyer, H.; Bossert, F.; Horstmann, H. *Liebigs Ann. Chem.* **1977**, *3*, 1895.
12. Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408.
13. Zaragoza, F.; Stephensen, H. *J. Org. Chem.* **2001**, *66*, 2518.
14. Clifton, A. D.; Young, P. R.; Cohen, P. *FEBS Lett.* **1996**, *392*, 209.
15. THP-1 cells were pre-incubated for 1 h with or without compound at the indicated concentrations and then stimulated with LPS or vehicle (basal condition) for 15 min. Total lysates were prepared and analyzed by Western blot, using an anti-phospho-hsp27 antibody (Upstate #05-645, dilution 1: 5000) and an anti-hsp27 antibody (Upstate #06-478, dilution 1: 1000). Bands were visualized with enhanced chemoluminescence (Amersham Pharmacia Biotech).
16. Some contribution of kinases upstream of p38 as well as involvement of JNK2 in the case of compound **17** cannot be fully excluded.