Bioorganic & Medicinal Chemistry Letters 19 (2009) 5136–5139

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Inhibitors of HIV-1 attachment. Part 3: A preliminary survey of the effect of structural variation of the benzamide moiety on antiviral activity ☆

Nicholas A. Meanwell [a,*], Owen B. Wallace [a], Henry Wang [a], Milind Deshpande [a], Bradley C. Pearce [a], Ashok Trehan [a], Kap-Sun Yeung [a], Zhilei Qiu [a], J. J. Kim Wright [a], Brett A. Robinson [b], Yi-Fei Gong [b], Hwei-Gene Heidi Wang [b], Wade S. Blair [b], Pei-Yong Shi [b], Pin-fang Lin [b]

[a] Department of Chemistry, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, United States
[b] Department of Virology, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, United States

ARTICLE INFO

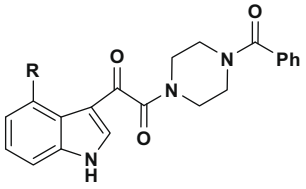
Article history:
Received 15 May 2009
Revised 23 June 2009
Accepted 2 July 2009
Available online 10 July 2009

Keywords:
HIV attachment inhibitor
Antiviral
Indole glyoxamide
HIV inhibitor

ABSTRACT

1-(4-Benzoylpiperazin-1-yl)-2-(1*H*-indol-3-yl)ethane-1,2-dione (**1a**) has been characterized as an inhibitor of HIV-1 attachment that interferes with the interaction of viral gp120 with the host cell receptor CD4. In previous studies, the effect of indole substitution pattern on antiviral activity was probed. In this Letter, the effect of structural variation of the benzamide moiety is described, a study that reveals the potential or the phenyl moiety to be replaced by five-membered heterocyclic rings and a restricted tolerance for the introduction of substituents to the phenyl ring.

We have recently described the preliminary structure–activity relationships associated with a series of HIV-1 inhibitors derived from the indole glyoxamide **1a**, a compound discovered by screening the Bristol-Myers Squibb compound collection using a pseudotype virus assay.[1–6] Mechanistic studies suggest that these compounds act by stabilizing a conformation of the HIV-1 glycoprotein gp120 that is poorly recognized by the host cell receptor CD4, thereby interfering with a specific cell attachment event, one of the initial steps in virus entry.[6–8] However, under different experimental conditions, these compounds appear to be capable of forming a ternary complex with gp120 and CD4 in a fashion that blocks CD4-mediated exposure of the gp41 fusion apparatus, providing an additional mechanistic facet.[9,10] In previous articles, we have described fundamental aspects of the structure–activity relationships (SAR) associated with the introduction of relatively simple substituents to the indole ring, a survey that established the patterns conferring optimal antiviral activity.[2] In this Letter, we describe the results of a complementary survey conducted after discovering **1a** in which the effect of structural variation of the benzamide moiety on HIV-1 inhibitory activity was probed. The objective of this exercise was straightforward and focused simply on defining the basic structural parameters required for optimal antiviral activity in a novel pharmacophore. Whilst the substrate selected for the initial phase of this work was the simple unsubstituted indole moiety found in **1a**, the discovery that the 4-fluoro derivative **1an** exhibited over 50-fold improved potency led to the inclusion of this indole moiety into the latter stages of the study.



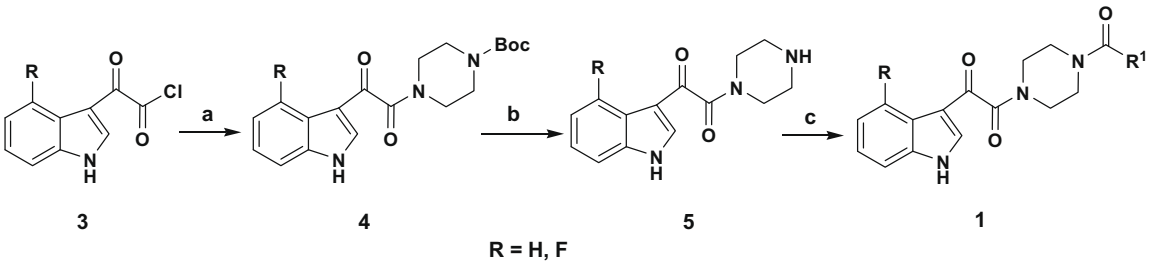
1a: R = H
1an: R = F

The target compounds were synthesized by the straightforward process depicted in Scheme 1. Coupling of 2-(1*H*-indol-3-yl)-2-oxoacetyl chloride or the 4-F derivative (**3**) with *tert*-butyl-1-piperazinecarboxylate in CH_2Cl_2 or THF using $i Pr_2NEt$ or Et_3N as the base afforded the piperazinamide **4**.[4,11] The Boc protecting element was removed from **4** by stirring with 20% CF_3CO_2H in CH_2Cl_2 and the resulting amine **5** coupled with a carboxylic acid using either 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) or the polymer-supported version of this reagent as the dehydrating agent in DMF to afford compounds **1a–1aag**, compiled in Table 1.

☆ See Refs. 1 and 2 for Parts 1 and 2 of this series.

* Corresponding author. Tel.: +1 203 677 6679.
E-mail address: Nicholas.Meanwell@bms.com (N.A. Meanwell).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.027



Scheme 1. Reagents and conditions: (a) *tert*-butyl-1-piperazinecarboxylate/*i*Pr_2NEt or Et_3N in CH_2Cl_2 or THF; (b) 20% CF_3CO_2H/CH_2Cl_2; (c) R^1CO_2H/1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) or polymer-supported 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (P-EDC)/DMF.

The antiviral properties of target compounds were assessed in a pseudotype virus infection system using an engineered virus. A proviral clone of LAI virus with the *env* gene replaced by the firefly luciferase gene[1–5] was co-transfected into HEK-293 cells with a plasmid expressing the envelope of the JRFL virus, a CCR5-specific virus. After 48 h, supernatant containing the recombinant pseudovirus, designated as JRFL-LucΔEnv, was harvested and the titer determined by performing serial dilutions in HeLa67 cells, which express the primary HIV-1 receptor CD4 and the coreceptor CCR5. Virus growth was quantified by measuring luciferase activity (Luciferase Gene Reporter Assay Kit by Roche) three days post-infection. For the analysis of the antiviral activity of test compounds, fourfold serial dilutions of compounds were added to JRFL-LucΔEnv-infected HeLa67 cells at the time of infection. After three days, the extent of luciferase activity was compared to controls where no compound was added and used to calculate the EC_{50} values for individual compounds. The cytotoxicity of test compounds was determined in parallel on HeLa67 cells and an XTT assay performed three days after compound addition. The results are presented in Table 1 where individual results are provided as a measure of assay variability when the data reported are the average of only two experiments.

The data presented in Table 1 provide essential insights into the structural requirements of the benzamide phenyl element in this series of HIV-1 attachment inhibitors. In the context of the simple indole **1a**, substitution of the benzamide phenyl ring with halogens, alkyl, alkoxy or dialkylamino moieties, examined in the context of compounds **1b–1u**, invariably resulted in reduced antiviral potency. Only the 2-F derivative **1b** maintained an EC_{50} of less than 1 μM in the pseudotype assay, with larger substituents generally providing compounds 20–200-fold weaker than the parent, irrespective of the site of deployment on the phenyl ring. This result is interpreted as a limited tolerance for sterically demanding substituents in this element of the pharmacophore, an aspect confirmed with the series of polysubstituted analogs **1v–1af** that focused specifically on assessing patterns of fluorine and chlorine substitution. Fusion of an additional ring (**1ah**) or homologation to the phenylacetic acid derivatives **1ag** and **1ao** provided inactive compounds whilst saturation of the phenyl ring (**1al**) resulted in a 22-fold reduction in antiviral potency that was further eroded by homologation, as probed with compound **1am**.

With these results in hand, attention was focused on exploring the effect of ring systems that would more subtly alter the silhouette cast by the phenyl ring. Within the pyridyl series captured by compounds **1ai, 1aj, 1ap–1ar**, a 2-pyridyl moiety is superior to the more exposed 3- and 4-isomers, best exemplified in the context of indole 4-fluoro-substitution where **1ap** exhibits potency approaching that of the prototype **1an**. That the potency of the 2-pyridyl derivative is largely retained in the context of the 4-fluoroindole series **1ap** but leads to a 10-fold reduction in the unsubstituted parent **1ai** may be a function, in part, of the intrinsically more potent background provided by the substituted indole analogue or the increased lipophilicity. A smaller furan ring is well tolerated, with the 2-furanyl derivative **1as** fourfold more potent than the 3-isomer **1aw**, and potent antiviral activity is preserved with the introduction of small substituents in the 2-substituted series (**1at–1av**). The 2- and 3-thienyl analogues **1ax** and **1aab** are also potent HIV-1 attachment inhibitors but activity in the former series is abrogated by 4-, 17- and 24-fold when Br (**1aaa**), Cl (**1az**) or CH_3 (**1ay**) substituents, respectively, are introduced at C-3. Within the series of azoles examined in the context of **1aac–1aag**, the 4-thiazole **1aaf** is optimal, with the more basic imidazolyl derivative **1aae** proving to be the weakest representative of this subset of compounds.

Collectively, these results define the scope of substitution tolerated by the benzamide element as somewhat limited, with the most effective structural modifications restricted to isosteric replacement of the phenyl ring by either pyridyl or select five-membered ring heterocycles.[12] Based on resistance mapping experiments, this series of HIV-1 attachment inhibitor is thought to bind to a conserved, recessed pocket within gp120 that recognizes Phe_{43} of CD4.[5–10,13,14] The sensitivity of the benzamide moiety to modification reflects the notion that this element of the pharmacophore may bind to a hydrophobic cavity of defined dimensions.[13,14] These structure–activity findings are similar to that reported for a related series of HIV-1 attachment inhibitors based on an indole-3-sulfonamide scaffold that appears to be inherently less potent and for which benzamide replacements were examined in a less systematic fashion.[15] Although we were unable to identify benzamide derivatives or replacements that offered markedly enhanced potency compared to the prototype **1a**, these results provided a clear definition of the optimal amide moieties. Consequently, the simple benzamide was included in BMS-377806 (**6**), an inhibitor of HIV-1 attachment that has been shown to protect macaques from vaginal SHIV challenge,[1,16] and BMS-488043 (**7**), a related compound that has demonstrated antiviral activity in early clinical trials conducted in HIV-1-infected subjects.[17–21] These studies have established the viability for this mechanistic class of HIV-1 inhibitor to function effectively in a preclinical model of prophylaxis and as a therapeutic agent in HIV-1 infected subjects.

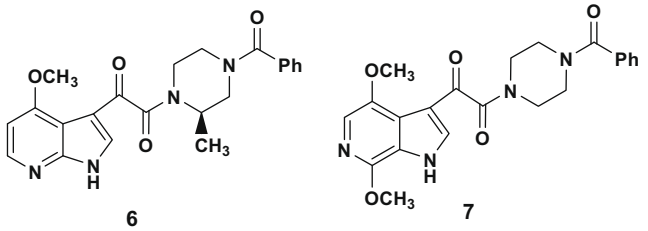
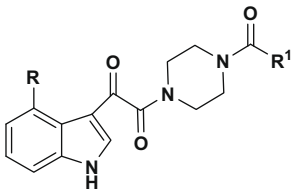


Table 1
Structure, HIV pseudotype virus inhibitory activity and cytotoxicity associated with indole glyoxamide derivatives **1a**–**1aag**.



Compound #	R	R^1	EC_{50}[a] (μM)	CC_{50} (μM)
1a	H	C_6H_5	0.153 ± 0.119 (*n* = 21)	338.8 ± 50.7 (*n* = 21)
1b	H	2-F–C_6H_4	0.391 ± 0.025 (*n* = 3)	101 (*n* = 1)
1c	H	2-Cl–C_6H_4	35 (*n* = 1)	>300 (*n* = 1)
1d	H	2-Br–C_6H_4	10.5 (*n* = 1)	>300 (*n* = 1)
1e	H	2-CH_3O–C_6H_4	>10 (*n* = 3)	>300 (*n* = 1)
1f	H	2-HO–C_6H_4	3.65 (*n* = 1)	194 (*n* = 1)
1g	H	2-$(CH_3)_2N$–C_6H_4	>0.50 (*n* = 2)	89.0 (82.4, 95.7)
1h	H	3-F–C_6H_4	2.76 ± 0.08 (*n* = 4)	200 (*n* = 1)
1i	H	3-Cl–C_6H_4	17.5 (*n* = 1)	117 (*n* = 1)
1j	H	3-Br–C_6H_4	6.70 ± 3.61 (*n* = 3)	76 (*n* = 1)
1k	H	3-CH_3O–C_6H_4	> 56.0 (*n* = 3)	>300 (*n* = 1)
1l	H	3-$(CH_3)_2N$–C_6H_4	>0.50 (*n* = 2)	230 (199.1, 260.9)
1m	H	4-F–C_6H_4	8.91 ± 5.63 (*n* = 3)	280 (*n* = 1)
1n	H	4-Cl–C_6H_4	65.80 ± 18.31 (*n* = 3)	92 (*n* = 1)
1o	H	4-Br–C_6H_4	30 (*n* = 1)	>240 (*n* = 1)
1p	H	4-CH_3O–C_6H_4	30.18 (31.75, 28.60)	129 (*n* = 1)
1q	H	4-HO–C_6H_4	2.49 ± 1.94 (*n* = 3)	136.1 ± 46.1 (*n* = 3)
1r	H	4-$(CH_3)_2N$–C_6H_4	>100 (*n* = 1)	>300 (*n* = 1)
1s	H	4-CH_3–C_6H_4	8.38 ± 4.3 (*n* = 3)	>99 (*n* = 3)
1t	H	4-*n*Pr–C_6H_4	82 (*n* = 1)	48 (*n* = 1)
1u	H	4-*t*Bu–C_6H_4	9 (*n* = 1)	31 (*n* = 1)
1v	H	2,4-Di-F–C_6H_3	6 (*n* = 1)	>300 (*n* = 1)
1w	H	3,4-Di-F–C_6H_3	8.3 (*n* = 1)	>300 (*n* = 1)
1x	H	2,6-Di-F–C_6H_3	6.7 (*n* = 1)	>300 (*n* = 1)
1y	H	2,4,5-Tri-F–C_6H_2	36.5 (*n* = 1)	>300 (*n* = 1)
1z	H	3,4,5-Tri-F–C_6H_2	55 (*n* = 1)	>300 (*n* = 1)
1aa	H	2,4,6-Tri-F–C_6H_2	>54 (*n* = 1)	>300 (*n* = 1)
1ab	H	2,3,4,5-Tetra-F–C_6H	>100 (*n* = 1)	>300 (*n* = 1)
1ac	H	2,3-Di-Cl–C_6H_3	18 (*n* = 1)	235 (*n* = 1)
1ad	H	2,4-Di-Cl–C_6H_3	37 (*n* = 1)	111 (*n* = 1)
1ae	H	3,4-Di-Cl–C_6H_3	14.5 (*n* = 1)	123 (*n* = 1)
1af	H	2-F,3-Cl–C_6H_3	3.5 (*n* = 1)	>300 (*n* = 1)
1ag	H	$C_6H_5CH_2$	>100 (*n* = 1)	>300 (*n* = 1)
1ah	H	1-Naphthyl	>100 (*n* = 1)	>300 (*n* = 1)
1ai	H	2-Pyridyl	1.55 (*n* = 1)	>300 (*n* = 1)
1aj	H	3-Pyridyl	4.2 (*n* = 1)	>300 (*n* = 1)
1ak	H	4-CH_3-3-Pyridyl	86.5 (*n* = 1)	>300 (*n* = 1)
1al	H	C_6H_{11}	3.5 (*n* = 1)	>300 (*n* = 1)
1am	H	$C_6H_{11}CH_2$	>100 (*n* = 1)	158 (*n* = 1)
1an	F	C_6H_5	0.0026 ± 0.0025 (*n* = 17)	>300 (*n* = 7)
1ao	F	$C_6H_5CH_2$	>50 (*n* = 1)	>300 (*n* = 1)
1ap	F	2-Pyridyl	0.0045 (0.0042, 0.0049)	>300 (*n* = 2)
1aq	F	3-Pyridyl	0.154 ± 0.071 (*n* = 3)	>300 (*n* = 3)
1ar	F	4-Pyridyl	0.0237 ± 0.006 (*n* = 3)	>205 (*n* = 1)
1as	F	2-Furanyl	0.0054 ± 0.0016 (*n* = 3)	>300 (*n* = 3)
1at	F	3-Cl-2-Furanyl	0.0031 ± 0.0017 (*n* = 4)	250.2 (250.3, 250.2)
1au	F	3-Br-2-Furanyl	0.0014 ± 0.0007 (*n* = 4)	236.4 (198.2, 274.6)
1av	F	3-CN-2-Furanyl	0.007 (0.008, 0.005)	187.3 (257.3, 117.4)
1aw	F	3-Furanyl	0.0204 ± 0.0116 (*n* = 4)	>300 (*n* = 4)
1ax	F	2-Thienyl	0.0007 ± 0.0005 (*n* = 3)	>300 (*n* = 1)
1ay	F	3-CH_3-2-Thienyl	0.0173 ± 0.0016 (*n* = 4)	260 (*n* = 1)
1az	F	3-Cl-2-Thienyl	0.0119 ± 0.001 (*n* = 4)	50.5 (43.4, 57.6)
1aaa	F	3-Br-2-Thienyl	0.0032 (0.0040, 0.0025)	69.7 (47.6, 91.8)
1aab	F	3-Thienyl	0.0004 ± 0.0003 (*n* = 4)	>300 (*n* = 2)
1aac	F	5-Isoxazolyl	0.1558 ± 0.032 (*n* = 3)	>300 (*n* = 3)
1aad	F	3-Pyrazolyl	0.0718 ± 0.027 (*n* = 3)	>300 (*n* = 3)
1aae	F	4-Imidazolyl	1.23 (1.50, 0.97)	>300 (*n* = 2)
1aaf	F	4-Thiazolyl	0.0087 ± 0.0034 (*n* = 3)	>250 (*n* = 1)
1aag	F	2-CH_3-4-thiazolyl	0.0228 ± 0.0124 (*n* = 4)	>300 (*n* = 2)

[a] Data are the means of two or more experiments, except where noted, with individual data provided for those experiments conducted twice.

References and notes

1. For Part 1 of this series see: Wang, T.; Zhang, Z.; Wallace, O. B.; Deshpande, M.; Fang, H.; Yang, Z.; Zadjura, L. M.; Tweedie, D. L.; Huang, S.; Zhao, F.; Ranadive, S.; Robinson, B.; Gong, Y.-F.; Riccardi, K.; Spicer, T. P.; Deminie, C.; Rose, R.; Wang, H.-W. H.; Blair, W. S.; Shi, P.-Y.; Lin, P.-F.; Colonno, R. J.; Meanwell, N. A. *J. Med. Chem.* **2003**, *46*, 4236.
2. For Part 2 of this series see: Meanwell, N. A.; Wallace, O. B.; Fang, H.; Wang, H.; Deshpande, M.; Wang, T.; Yin, Z.; Zhang, Z.; Pearce, B. C.; James, J.; Yeung, K.-S.; Qiu, Z.; Wright, J. J. K.; Yang, Z.; Zadjura, L.; Tweedie, D. L.; Yeola, S.; Zhao, F.; Ranadive, S.; Robinson, B. A.; Gong, Y.-F.; Wang, H.-G. H.; Blair, W. S.; Shi, P.-Y.; Colono, R. J.; Lin, P.-F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1977.
3. Blair, W., Spicer, T. P. World Patent Application, WO-2001/96610, December 20, 2001.
4. Blair, W. S.; Deshpande, M.; Fang, H.; Lin, P.-F.; Spicer, T. P.; Wallace, O. B.; Wang, H.; Wang, T.; Zhang, Z.; Yeung, K.-S. World Patent Application, WO2000/76521, December 21, 2000.
5. Lin, P.-F.; Blair, W.; Wang, T.; Spicer, T.; Guo, Q.; Zhou, N.; Gong, Y.-F.; Wang, H.-W. H.; Rose, R.; Yamanaka, G.; Robinson, B.; Li, C.-B.; Fridell, R.; Deminie, C.; Demers, G.; Yang, Z.; Zadjura, L.; Meanwell, N.; Colonno, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11013.
6. Wang, H.-W. H.; Williams, R. E.; Lin, P.-F. *Curr. Pharm. Des.* **2004**, *10*, 1785.
7. Guo, Q.; Ho, H.-T.; Dicker, I.; Fan, L.; Zhou, N.; Friborg, J.; Wang, T.; McAuliffe, B. V.; Wang, H.-W. H.; Rose, R. E.; Fang, H.; Scarnati, H. T.; Langley, D. R.; Meanwell, N. A.; Abraham, R.; Colonno, R. J.; Lin, P.-f. *J. Virol.* **2003**, *77*, 10528.
8. Ho, H.-T.; Nowicka-Sans, B.; McAuliffe, B.; Li, C.-B.; Yamanaka, G.; Zhou, N.; Fang, H.; Dicker, I.; Dalterio, R.; Gong, Y.-F.; Wang, T.; Yin, Z.; Ueda, Y.; Matiskella, J.; Kadow, J.; Clapham, P.; Robinson, J.; Colonno, R.; Lin, P.-F. *J. Virol.* **2006**, *80*, 4017.
9. Si, Z.; Madani, N.; Cox, J. M.; Chruma, J. J.; Klein, J. C.; Schön, A.; Phan, N.; Wang, L.; Biorn, A. C.; Cocklin, S.; Chaiken, I.; Freire, E.; Smith, A. B., III; Sodroski, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5036.
10. Madani, N.; Perdigoto, A. L.; Srinivasan, K.; Cox, J. M.; Chruma, J. J.; LaLonde, J.; Head, M.; Smith, A. B., III; Sodroski, J. G. *J. Virol.* **2004**, *78*, 3742.
11. Lingens, F.; Lange, J. *Justus Liebigs Ann. Chem.* **1970**, *738*, 46.
12. Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.
13. Kong, R.; Tan, J. J.; Ma, X. H.; Chen, W. Z.; Wang, C. X. *Biochim. Biophys. Acta* **2006**, *1764*, 766.
14. Teixeira, C.; Serradji, N.; Maurel, F.; Barbault, F. *Eur. J. Med. Chem.* **2009**. doi:10.1016/j.ejmech.2009.03.028.
15. Lu, R.-J.; Tucker, J. A.; Zinevitch, T.; Kirichenko, O.; Konoplev, V.; Kuznetsova, S.; Sviridov, S.; Pickens, J.; Tandel, T.; Brahmachary, E.; Yang, Y.; Wang, J.; Freel, S.; Fisher, S.; Sullivan, A.; Zhou, J.; Stanfield-Oakley, S.; Greenberg, M.; Bolognesi, D.; Bray, B.; Koszalka, B.; Jeffs, P.; Khasanov, A.; Ma, Y.-A.; Jeffries, C.; Liu, C.; Proskurina, T.; Zhu, T.; Chucholowski, A.; Li, R.; Sexton, C. *J. Med. Chem.* **2007**, *50*, 6535.
16. Veazey, R. S.; Klasse, P. J.; Schadr, S. M.; Hu, Q.; Ketas, T. J.; Lu, M.; Marx, P. A.; Dufour, J.; Colonno, R. J.; Shattock, R. J.; Springer, M. S.; Moore, J. P. *Nature* **2005**, *438*, 99.
17. Hanna, G.; Lalezari, J.; Hellinger, J.; Wohl, D.; Masterson, T.; Fiske, W.; Kadow, J.; Lin, P.-F.; Giordano, M.; Colonno, R.; Grasela D. In *11th Conf. Retroviruses Opportunistic Infections, San Francisco*, February 8–11, 2004, Abstract 141.
18. Doms, R. W.; Trono, D. *Genes Dev.* **2000**, *14*, 2677.
19. Biscone, M. J.; Pierson, T. C.; Doms, R. W. *Curr. Opin. Pharmacol.* **2002**, *2*, 529.
20. Kadow, J.; Wang, H.-G. H.; Lin, P.-F. *Curr. Opin. Invest. Drugs* **2006**, *7*, 721.
21. Strizki, J. *Adv. Pharmacol.* **2008**, *56*, 93.