Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 653–656
Bioorganic & Medicinal Chemistry Letters

Novel heterocycle-substituted pyrimidines as inhibitors of NF-κB transcription regulation related to TNF-α cytokine release

Hyung-Ho Ha, Jee Seon Kim and B. Moon Kim*

Department of Chemistry, Seoul National University, Seoul 151-747, Republic of Korea

Received 9 August 2007; revised 29 October 2007; accepted 17 November 2007
Available online 22 November 2007

Abstract—Novel heterocyclic ring-substituted pyrimidines have been designed as inhibitors of glycogen synthase kinase-3β (GSK-3β) from the modification of known inhibitors. Several potent inhibitors exhibiting nanomolar activities were discovered against GSK-3β kinase as well as in an NF-κB reporter gene assay. Based on the results from in vitro TNF-α release inhibition and in vivo endotoxima, these inhibitors are expected to be useful candidates for treatment of inflammation-related diseases.

The transcription nuclear factor κB (NF-κB)[1] is a pivotal regulator of the inducible expression of key pro-inflammatory mediators. Activated NF-κB has been observed in several debilitating inflammatory disorders including rheumatoid arthritis (RA)[2] and osteoarthritis. In activated T cells, transcription factors such as the activator protein-1 (AP-1)[3] regulate IL-2 and matrix metalloproteinase production, while the NF-κB is essential for the transcriptional regulation of the pro-inflammatory cytokines[4] IL-1, IL-6, IL-8, and TNF-α. Though the mechanism for the inhibition of NF-κB is well established,[1] complications which depend on the signal inducing molecules, cell types, and target genes are still waiting to be deciphered. However, the IκB protein family members are the most promising classical target of inhibition for NF-κB. The IκB kinase (IKK) complex is the primary mediator of IκB phosphorylation and is activated by various stimuli including cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 (IL-1).

The glycogen synthase kinase-3β (GSK-3β)[5] is a critical factor in the regulation of a wide variety of signaling proteins and transcription factors, including cyclin D1, c-Jun, NF-ATc, and β-catenin, among others. Numerous reports have implicated GSK-3β in the control of various signaling pathways that activate NF-κB, including regulation of NF-κB1/p105 stability[6] as well as IKK activity. However, NF-κB DNA binding and luciferase reporter assay activity were reduced in the GSK-3β null cells, which is consistent with the report that GSK-3β is able to affect NF-κB function downstream of release from IκBβ. Recently, a number of reports have emerged describing molecules that inhibit GSK-3β targeting mostly diabetes and Alzheimer disease.[7]

However, a recent publication has demonstrated that TNF-induced NF-κB reporter gene transcription was also suppressed in GSK-3β gene-deleted cells.[8,9] GSK-3β was also reported to inhibit the systemic inflammation of in vivo endotoxic model induced by LPS,[10] while another study has demonstrated that inhibition of GSK-3β potentiated TNF-induced expression of IL-6 and MCP-1 by 2–6-fold.[11] Generally TNF-α contributes to the regulation of the body's response to infection and cellular stress. Chronic and excessive production of TNF-α are believed to underlie the progression of many autoimmune diseases such as rheumatoid arthritis (RA), Chrohn's disease, and psoriasis.[12]

GlaxoSmithKline researchers reported pyrazolo[3,4-*b*]pyridines represented by compound **1** as potent inhibitors of GSK-3 and they showed very promising kinase inhibitor activities (Fig. 1).[13] Based upon our preliminary modeling studies of the GlaxoSmithKline structures, two possible structural modifications were envisioned, namely structures A and B in Figure 1.

In structure A, the 6-position of the pyridine ring appeared to have some room for extra binding.[14] Fixing

Keywords: Glycogen synthase kinase 3β; Nuclear factor κB; Anti-inflammation; TNF-α cytokine; Heterocyclic fused ring.
* Corresponding author. Tel.: +82 2 880 6644; fax: +82 2 872 7505; e-mail: kimbm@snu.ac.kr

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.064

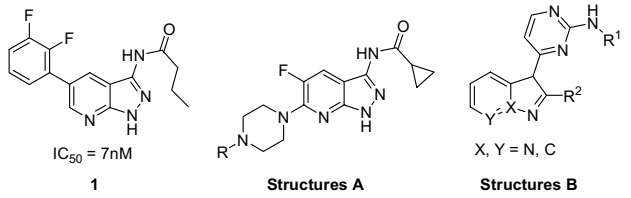


Figure 1. New inhibitor design based upon the GlaxoSmithKline inhibitor **1** against GSK-3.

cyclopropanecarboxamide for 3-amino group and a small fluoride group at the 5 position, we focused on the diversification at the 6-position of the bicyclic ring with piperazine derivatives. Derivatives of the structure A were prepared as outlined in Scheme 1.

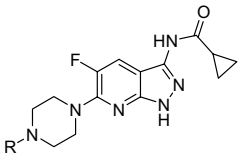
In Table 1 in vitro activity results from this modification are listed. All five compounds tested were found to be inactive for GSK-3β kinase and also for the reporter gene assay of NF-κB, and AP-1 showed moderate and unsatisfactory results, respectively.

From the above results, it was not possible to draw any conclusion on the relationship between GSK-3β and NF-κB transcription. We then turned our attention to targeting GSK-3 inhibition using structure B.

Diversity in the structure B was given in three parts; R_1, R_2, and the heteroatom (X and/or Y) in the bicyclic ring. Compounds **12**–**16** were prepared through the sequence of reactions shown in Scheme 2. Pyrazolopyridine ring formation and aminopyrimidine synthesis, two key synthetic reactions in this scheme, proceeded smoothly.

The R_2 group was fixed as H or phenyl as shown in Scheme 2. However, for R_1, broader diversity was introduced and from this primary SAR study three aniline derivatives were selected. In Scheme 3 a reaction sequence is depicted for the construction of pyrolopyridine or indole core structures. Methoxyethyl group[15] was introduced for the enhancement of physicochemical property of the inhibitors which often exhibit high lipophilicity (Table 2).

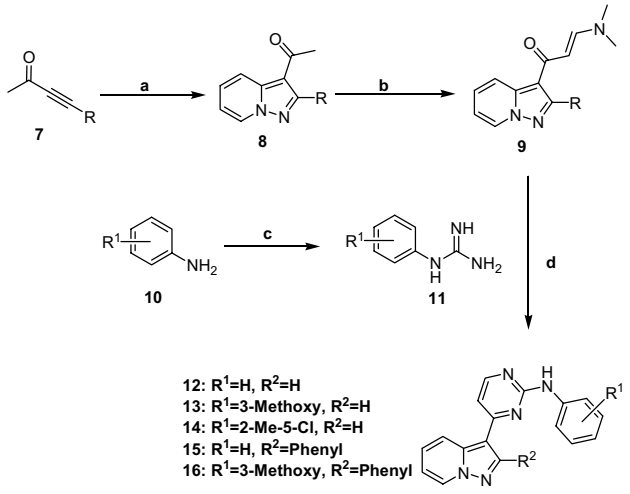
Table 1. Inhibition of pyrazolopyridine analogues against GSK-3β, and NF-κB and AP-1 reporter gene assay results for compounds **2**–**6**



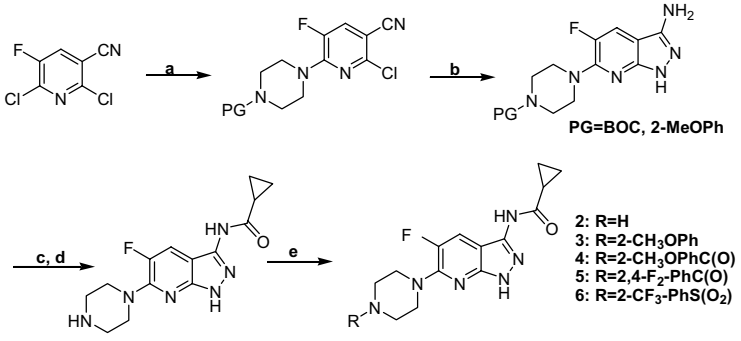
Compound	R	GSK-3β, IC_{50}[a] (μM)	NF-κB, IC_{50}[a] (μM)	AP-1[b], IC_{50}[a] (μM)
2	H	na	na	na
3	2-CH_3OPh	na	10.9 (±0.5)	na
4	2-CH_3OPhC(O)	na	13.1 (±1.2)	na
5	2,4-F_2-PhC(O)	na	19.3 (±4.6)	na
6	2-CF_3-PhS(O_2)	na	9.4 (±0.8)	na

[a] Values are means of three experiments, standard deviation is given in parentheses (na, not active).

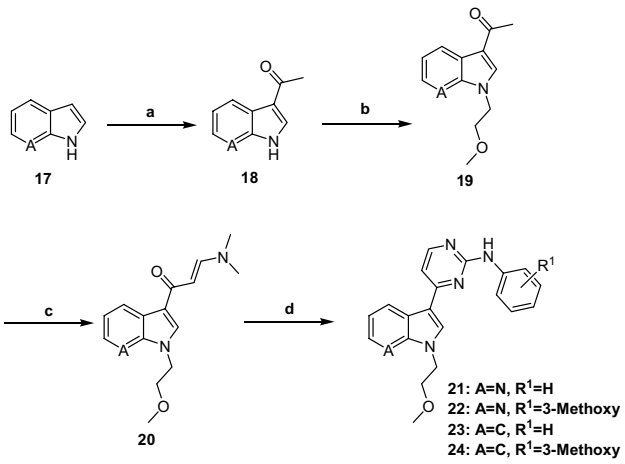
[b] In vitro therapeutic index (IC_{50} cytotoxicity/IC_{50} complement inhibition).



Scheme 2. A general synthetic process for structure B. Reagents and conditions: (a) 1-aminopyridinium iodide, KOH, H_2O, rt, 60–70%; (b) DMF–DMA, DMF, 130 °C, 65–75%; (c) conc nitric acid, 50% cyanamide, ethanol, 90 °C, 82%; (d) K_2CO_3, 2-methoxyethanol, 120 °C, 40–50%.



Scheme 1. A general synthetic scheme for structure A. Reagents and conditions: (a) piperazine, Et_3N, CH_3CN, refluxed 2 h, 94–96%; (b) hydrazine hydrate, 2-methoxyethanol, refluxed 6 h, 82–86%; (c) cyclopropanecarbonyl chloride, pyridine, refluxed 4 h, 80–84%; (d) 4 N-HCl in dioxane, rt 5 h, 96%; (e) RCOCl or RSO_3Cl, Et_3N, CH_2Cl_2, 82–86%.



Scheme 3. A general synthetic process for compounds **21**–**24**. Reagents and conditions: (a) $AlCl_3$, AcCl, CH_2Cl_2, rt, 76–85%; (b) 2-bromoethyl methyl ether, K_2CO_3, DMF, 50 °C, 80–85%; (c) DMF–DMA, DMF, 130 °C, 75–80%; (d) guanidine, *t*-BuOK, THF, 80 °C, 50–55%.

The main difference in structures A and B is the aminopyrimidine moiety. Generally this structural motif has been well known for kinase inhibitor activity. A few groups tried to combine this fragment and the pyrazolo-pyridine moiety to increase activity and selectivity for kinase and antiviral inhibition.[16] Binding mode of the inhibitors featuring the two fragments combined was predicted through in silico modeling as depicted in Figure 2.[17] According to the modeling studies of compound **21**, phenylaminopyrimidine group appears to have a unique interaction with Tyr134 and Val135 in the hydrophobic pocket. The binding appears to be optimal with the pyrimidyl-substituted bicyclic ring. The result of inhibition against GSK-3 could be analyzed in two aspects, namely, the core structure and peripheral substitution. Pyrolo-pyridines **12**–**16** showed better activities not only in the GSK-3β kinase assay[18] but also in the NF-κB reporter gene assay compared to

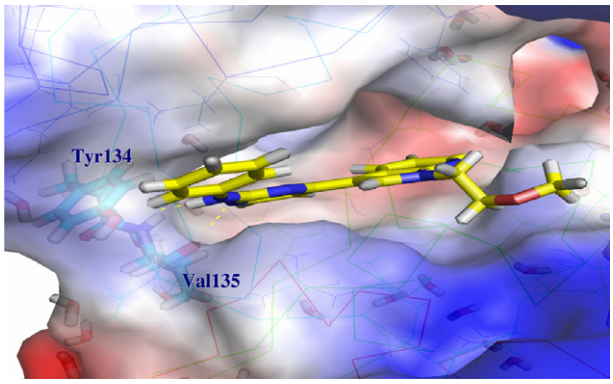


Figure 2. In silico surface model of the GSK-3 ATP-binding pocket from PDB database with compound **21**.

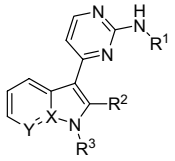
compounds **21**–**24**. The R^1 substitution did not appear to contribute much toward the activity except for the compounds **13** and **14**, where a pronounced difference toward GSK-3β was observed. However, the phenyl substitution at R^2 showed a dramatic increase in activity. It appears that a major contribution for the activity enhancement was from the aromatic nitrogen. To obtain selective compounds for inflammation related mainly with NF-κB, three compounds were selected and tested for the inhibition[19] of TNF-α production using (LPS)-stimulated human monocytic cells (THP-1) by ELISA and the data are shown in Table 3.

Table 3. In vitro functional assay for the inhibition of TNF-α release in THP-1 cell line

Compound:	3	12	22
TNF-α, IC_{50}[a] (μM)	na	0.14 (±0.02)	0.99 (±0.25)

[a] Values are means of three experiments, standard deviation is given in parentheses (na, not active).

Table 2. Results of the inhibition of aminopyrimidine analogues for GSK-3β, NF-κB, and AP-1 reporter gene assay for compounds **12–24**



Compound	R^1	R^2	R^3	X,Y	GSK-3β, IC_{50}[a] (μM)	NF-κB[b], IC_{50}[a] (μM)	AP-1[c], IC_{50}[a] (μM)
12	Ph	H	H	N,C	0.15 (±0.02)	0.6 (±0.05)	0.9 (±0.03)
13	3-CH_3OPh	H	H	N,C	0.10 (±0.02)	1.0 (±0.08)	1.8 (±0.08)
14	2-CH_3, 5-ClPh	H	H	N,C	10.60 (±1.08)	4.3 (±0.3)	4.9 (±0.7)
15	Ph	Ph	H	N,C	0.06 (±0.01)	1.7 (±0.1)	0.7 (±0.2)
16	3-CH_3OPh	Ph	H	N,C	0.03 (±0.14)	1.9 (±0.1)	1.6 (±0.3)
21	Ph	H	C_2H_4OMe	C,N	1.05 (±0.08)	5.6 (±0.6)	11.4 (±1.5)
22	3-CH_3OPh	H	C_2H_4OMe	C,N	2.20 (±0.07)	1.4 (±0.1)	na
23	Ph	H	C_2H_4OMe	C,C	0.32 (±0.13)	10.4 (±1.1)	na
24	3-CH_3OPh	H	C_2H_4OMe	C,C	0.14 (±0.18)	7.8 (±0.5)	na

[a] Values are means of three experiments, standard deviation is given in parentheses (na, not active).
[b] Inhibition of NF-κB mediated transcriptional activation in A-549 cells.
[c] Inhibition of AP-1 mediated transcriptional activation in Jurkat cells.

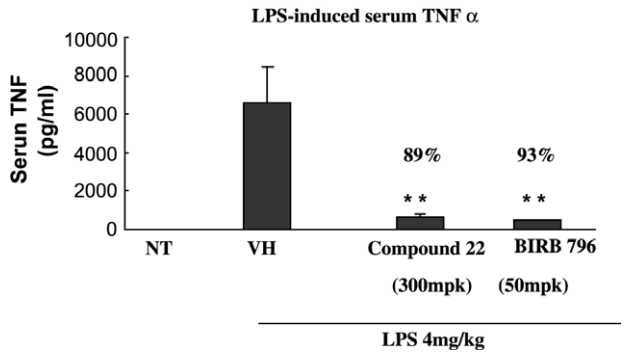


Figure 3. In vivo efficacy of compound **22** for serum TNF-α level after LPS treatment.

Not surprisingly, compound **3** was not active in the functional assay; however, compounds **12** and **22** exhibited good activities. Based on this result, it was clear that controlling cytokine levels involves disruption of the signal transduction pathway leading to their release from stimulated inflammatory cells by way of GSK-3β kinase and NF-κB transcription (Fig. 3).

Compound **22** was examined for inhibition[20] of TNF-α release in Balb/C mice challenged with LPS. It inhibited TNF-α level dramatically at 300 mg/kg dose compared to a reference compound,[21] p38 Map kinase inhibitor BIRB796.[22] Further in vivo efficacy study on this series of compounds is in progress.

In conclusion, we report a series of fused heterocyclic GSK-3 inhibitors showing nanomolar potency against GSK-3β, and submicromolar activities toward NF-κB and LPS-stimulated human monocytic cells (THP-1). From this result, in vitro TNF-α release inhibition and in vivo sepsis model could be used to identify promising candidates for treatment of inflammation related diseases.

Acknowledgment

We are grateful to Choongwae Pharmaceuticals for running the reporter gene assay and TNF-α release assay.

References and notes

1. For recent reviews, see (a) Hayden, M. S.; Ghosh, S. *Genes Dev.* **2004**, *18*, 2195; (b) Manning, A. M. *Drug Discov. Today* **1996**, *1*, 151.
2. Smolen, J. S.; Seiner, G. *Nat. Rev. Drug Discov.* **2003**, *2*, 473.
3. O'Shea, J. J.; Ma, A.; Lipsky, P. *Nat. Rev. Immunol.* **2002**, *2*, 37.
4. McCulloch, C. A.; Downey, G. P.; El-Gabalawy, H. *Nat. Rev. Drug Discov.* **2006**, *5*, 864.
5. (a) Jope, R. S.; Johnson, G. V. W. *Trends Biochem. Sci.* **2004**, *29*, 95; (b) Meijer, L.; Flajolet, M.; Greengard, P. *Trends Pharmacol. Sci.* **2004**, *25*, 471.
6. Demarchi, F.; Bertoli, C.; Sandy, P.; Schneider, C. *J. Biol. Chem.* **2003**, *278*, 39583.
7. Huang, H. C.; O'Brien, W. T.; Klein, P. S. *Drug Discov. Ther. Strateg.* **2006**, *3*, 613.
8. Martinez, A.; Alonso, M.; Castro, A.; Pe'rez, C.; Moreno, F. J. *J. Med. Chem.* **2002**, *45*, 1292.
9. Takada, Y.; Fang, X.; Jamaluddin, M. S.; Boyd, D. D.; Aggarwal, B. B. *J. Biol. Chem.* **2004**, *279*, 39541.
10. Whittle, B. J. R.; Varga, C.; Posa, A.; Molnar, A.; Collin, M.; Thiemermann, C. *J. Pharmacol.* **2006**, *147*, 575.
11. Angela, V.; Sientay, C.; Ira, G.; Uday, S.; Sivaram, P. *J. Biol. Chem.* **2006**, *281*, 16985.
12. Palladino, M. A.; Bahjat, F. R.; Theodorakis, E. A.; Moldawer, L. L. *Nat. Rev. Drug Discov.* **2003**, *2*, 736.
13. Witherington, J.; Bordas, V.; Garland, S. L.; Hickey, D. M. B.; Ife, R. J.; Liddle, J.; Saunders, M.; Smith, D. G.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1577.
14. Witherington, J.; Bordas, V.; Gaiba, A.; Garton, N. S.; Naylor, A.; Rawlings, A. D.; Slingsby, B. P.; Smith, D. G.; Takle, A. K.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3055.
15. Fonquerna, S.; Miralpeix, M.; Pages, L.; Puig, C.; Cardus, A.; Anton, F.; Cardenas, A.; Vilella, D.; Aparici, M.; Calaf, E.; Prieto, J.; Gras, J.; Huerta, J. M.; Warrellow, G.; Beleta, J.; Ryder, H. *J. Med. Chem.* **2004**, *47*, 6326.
16. Allen, S. H.; Johns, B. A.; Gudmundsson, K. S.; Freeman, G. A.; Boyd, F. L.; Sexton, C. H.; Selleseth, D. W.; Creech, K. L.; Moniri, K. L. *Bioorg. Med. Chem.* **2006**, *13*, 944.
17. The molecular modeling was performed with the Maestro software package from Schrodinger. The ligand was manually docked into the active site. Structural coordinates for the GSK-3 structure were taken from: Bax, B.; Carter, P. S.; Lewis, C.; Guy, A. R.; Bridges, A.; Tanner, R.; Pettman, G.; Mannix, C.; Culbert, A. A.; Brown, M. J. B.; Smith, D. G.; Reith, A. D. *Structure* **2001**, *9*, 1143.
18. GSK-3β was assayed in 96-well microtiter plates at a final concentration of 20 nM in 100 mL Hepes at pH 7.2 containing 10 mM $MgCl_2$, 0.1 mg/mL bovine serum albumin, 1 mM dithiothreitol, 0.3 mg/mL heparin, 2.8 M peptide substrate (Biotin-Ahx-AAAKRREILSRRP-S(PO3)YR-amide), 2.5 M ATP, and 0.2 Ci/well [^{33}P]ATP. After 40 min, the reaction was stopped by addition of 100 mM EDTA and 1.0 mM ATP, solution in 100 mM Hepes followed by a solution of streptavidin coated SPA beads (Amersham) in PBS to give a final concentration of 0.25 mg of beads per assay well. The plates were counted on a Packard TopCount NXT microplate counter.
19. Human monocytic cell line (THP-1) TNF-α release assay; THP-1 cells were seeded into 96-well plates at 1×10^6 cells/mL (200 L/well) in medium containing FCS (1%) and incubated overnight. Following pretreatment with compounds for 1 h, the cells were incubated with LPS (20 g/mL) for a further 24 h. TNF-α release was measured in the supernatants by sandwich ELISA. IC_{50} values shown from repeat experiments are means ($n = 3$).
20. Mouse TNF-α release assay; compound was administered orally to balb/c mice 30 min prior to LPS (0.1 mg/kg ip) challenge. Serum TNF-α levels were determined 90 min after LPS insult. Results represent means ($n = 3$).
21. Regan, J.; Breitfelder, S.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Hickey, E.; Klaus, B.; Madwed, J.; Moriak, M.; Moss, N.; Pargellis, C.; Pav, S.; Proto, A.; Swinamer, A.; Tong, L.; Torcellini, C. *J. Med. Chem.* **2002**, *45*, 1292.
22. Chen, Z.; Gibson, T. B.; Robinson, F.; Silvestro, L.; Pearson, G.; Xu, B.-e.; Wright, A.; Vanderbilt, C.; Cobb, M. H. *Chem. Rev.* **2001**, *101*, 2449.