Bioorganic & Medicinal Chemistry Letters 18 (2008) 3578–3581

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

2,5-Diaminopyrimidines and 3,5-disubstituted azapurines as inhibitors of glycogen synthase kinase-3 (GSK-3)

Christopher Lum [a,*], Jeff Kahl [b], Linda Kessler [c], Jeff Kucharski [c], Jan Lundström [a], Stephen Miller [d], Hiroshi Nakanishi [e], Yazhong Pei [f], Kent Pryor [a], Edward Roberts [g], Lubomir Sebo [h], Robert Sullivan [i], Jan Urban [j], Zhijun Wang [f]

[a] Kemia, Inc., 5871 Oberlin Dr., Ste. 100, San Diego, CA 92121, USA
[b] Azaya Therapeutics, 12500 Network Blvd. #207, San Antonio, TX 78249, USA
[c] Intellikine, 10931 North Torrey Pines Road, Suite 103, La Jolla, CA 92037, USA
[d] Array Biopharma, 3200 Walnut Street, Boulder, CO 80301, USA
[e] Center for Stem Cell and Regenerative Medicine, Keck School of Medicine, University of Southern California, 1501 San Pablo Street, ZNI-513B, Los Angeles, CA 90033, USA
[f] CalciMedica, 505 Coast Blvd S. Suite 209, La Jolla, CA 92037, USA
[g] The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
[h] Pacific Biosciences, 1505 Adams Drive, Menlo Park, CA 94025, USA
[i] Amylin Pharmaceuticals, Inc., 9360 Towne Centre Drive, San Diego, CA 92121, USA
[j] Nanosyn, 11558 Sorrento Valley Rd., San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 8 February 2008
Revised 25 April 2008
Accepted 1 May 2008
Available online 6 May 2008

Keywords:
Kinase inhibitors
GSK-3
Aurora A
Diabetes
Alzheimer's
Depression
Bipolar disorder
Molecular modeling

ABSTRACT

The discovery of two classes of pyrimidine-based inhibitors of GSK-3 is described. Optimization of these series led to inhibitors with IC_{50} < 10 nM and >100-fold selectivity over Aurora A kinase. A proposed binding mode of **21b** is presented. One compound (**33**) of the pyrimidine series showed promising pharmacokinetic parameters.

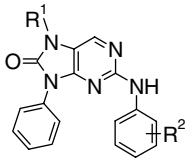
Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase which was first discovered for its role in phosphorylation-mediated deactivation of glycogen synthase, the rate-limiting enzyme in glycogen biosynthesis. Subsequently, GSK-3 has been found to be ubiquitously distributed throughout the body and to play a central role in many cellular and physiological events, including Wnt and Hedgehog signaling, transcription, insulin action, neuronal function and many others.[1] As a result, inhibition of GSK-3 has emerged as a potential therapeutic approach for a number of pathologies including Alzheimer's disease, bipolar disorders, and type II diabetes.[2] GSK-3 exists in two forms, α and β, which share 85% homology (95% in the catalytic domain), but which have shown distinct pharmacology.[1,3] We here report the identification and SAR evolution of two classes of molecules which are potent GSK-3 inhibitors and show good selectivity against other targets.

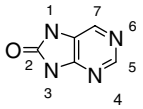
We obtained our initial hit **1** (Table 1) from screening our kinase compound collection.[4] Modifications based on the purinone scaffold failed to produce significant gains in potency. For example, reducing the size of the *N*-alkyl substituent in **2**–**4** yielded no improvements. However, removing the alkyl group entirely in **6** eliminated activity. Substitutions at the para position on the 5-arylamino ring with a carboxylic acid once again led to compounds in the micromolar range. However, in the course of screening the synthetic intermediates of these purinones, we observed that the nitropyrimidine **10** (Table 2) was also active. In contrast to the initial template, however, substitutions on the nitropyrimidine 2-arylamino group produced significant improvements in potency, particularly with carboxylic acid groups (Table 4, compounds

* Corresponding author. Tel.: +1 858 2434338.
E-mail address: chrislum@sbcglobal.net (C. Lum).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.001

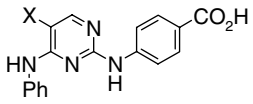
Table 1
GSK-3α and GSK-3β assay results for compounds **1–9**

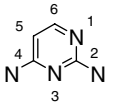




Compound #	R^1	R^2	GSK-3α %inh, 10 μM	GSK-3β %inh, 10 μM	GSK-3β IC_{50} (μM)
1	*t*-Bu	H	88	93	2.4
2	*i*-Pr	H		71	2.9
3	Et	H	73	78	>10
4	Me	H	94	96	1.7
5	cHx	H	16	14	
6	H	H	−7	20	
7	H	4-CO_2*n*-Bu	6	−5	
8	H	4-CO_2H	93	88	4.0
9	H	3-CO_2H	1	3	

Table 2
GSK-3α and GSK-3β assay results for compounds **10–15**





Compound #	X	GSK-3α IC_{50} (μM)	GSK-3β IC_{50} (μM)
10	NO_2	0.27	0.15
11	Br	82%[a]	3.20
12	4-Pyridyl	1.87	0.54
13	5-Triazolyl	2.85	1.17
14	CF_3	0.75	0.49
15	F	3.76	4.17

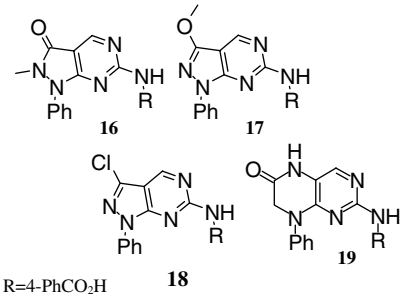
[a] % inhibition at 10 μM.

25a, **28a**, **29a**, and **30a**). Nitropyrimidines have been reported in a number of recent patents as inhibitors of kinases such as PKC-θ, CDKs, CHK, and others.[5–8] Because the aryl nitro moiety poses a risk for toxic metabolites, we attempted to find a replacement. However, with a set of groups which varied in size as in **11–15**, potency was reduced appreciably in all cases. Therefore, despite their potential liabilities the synthetic ease of generating nitropyrimidine derivatives made these compounds a valuable information-gathering tool for SAR exploration that could be applied to other templates.

In our search for a scaffold which would match the improved potency of the nitropyrimidine we initially looked into other fused ring systems **16–19** as replacement for the purinone (Table 3). Compound **18** showed a distinct potency advantage despite a close steric similarity with **16** and **17**. Although **18** contained a reactive functional group, it provided us with some useful information moving forward. In combination with the nitropyrimidine results, we observed that potency rank order correlated inversely to the electron density of the pyrimidine portion of the molecule.

This directed our design toward electron-poor substituents on the pyrimidine ring. A survey of the Hammett constants of common aromatic substitutions revealed the diazonium ion as the most electron-poor substituent available ($\sigma_p = 1.93$).[9] To that effect, the azapurine **20b** (Table 4) was envisioned, which is the result of the intramolecular reaction of the diazonium moiety with the adjacent amino group to form a 5,6-fused ring system. The enhanced electron-withdrawing effect of this fused triazole on the

Table 3
GSK-3α and GSK-3β assay results for compounds **16–19**



R=4-PhCO_2H **18**

Compound #	GSK-3α IC_{50} (μM)	GSK-3β IC_{50} (μM)
16	1.02	0.96
17	1.90	0.76
18	0.35	0.18
19	6.7	3.5

pyrimidine ring is supported by the proton NMR shifts. For example, the C-7-H shift in **20b** appears at δ 9.20 (DMSO-d_6), compared to δ 9.15 and δ 8.2 for the analogous protons in the nitropyrimidine **20a** and purinone **8**, respectively. Compounds of this class showed even greater potency than the nitropyrimidine, as evidenced by the 4-fold improvement over **20a** in the GSK-3β potency. Compound **20b** was submicromolar in potency even in the absence of the carboxylic acid substituent.

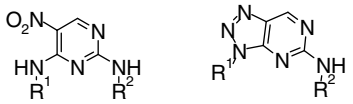
Docking models of **20b** into PDB structure 1q5k of GSK-3β[10] (Fig. 1) suggested that the N-6 and the 5-NH form hydrogen bonds to the hinge region in GSK-3β at the Val-135 amide and carbonyl, respectively. These correspond to hydrogen bonds at the N-1 and the 2-NH of the nitropyrimidines. We hypothesize that the enhanced potency of this class is due to polarization of the C-7-H bond so that the hydrogen can participate in a third hydrogen bond to the carbonyl of Asp-133. This polarization is supported by the aforementioned proton NMR shifts. C–H···O hydrogen bonds of this nature have been described previously in protein ligand interactions and supported by ab initio calculations.[11]

Although we now had a series with potencies consistently in the submicromolar range, the potential issues associated with carboxylic acids such as low blood–brain barrier permeability were compounded by cross-reactivity to Aurora A, revealed through selectivity screens. Docking models suggested that the enhanced potency of the carboxyl substituted phenyl ring were due to a charge–charge interaction with Arg141 on the C-lobe at the entrance to the GSK-3β ATP pocket (Fig. 1). We hypothesized that the lack of selectivity was due a similar interaction with the Lys171 of Aurora, which is in the homologous region. We speculated that replacing the carboxylic acid moieties with hydrogen bond donors in specific orientations would avoid the non-specific charge–charge interactions and take advantage specific hydrogen bonds to the Arg141 in GSK-3β. To that effect **23b**, **27b**, **32a**, and **32b** were synthesized and shown to retain potency on GSK-3α and β with good selectivity against Aurora A.

During the course of our studies, patents emerged outlining the use of azapurines in the inhibition of GSK-3.[12–15] A subset of our compounds containing aliphatic rings or bicyclic systems at the 5-amino position, or bridged rings at N-3 (exemplified by **23b**, **27b**, and **28b**) allowed us some freedom to operate, so we decided to focus on these series.

Docking models of **20b** also suggest that the substituent at the azapurine 3-position resides in the hydrophobic pocket normally

Table 4
GSK-3α and GSK-3β assay results for compounds **20–32**



20a-32a **20b-32b**
A **B**

Compound #	R^1	R^2	GSK-3α IC_{50} (nM)	GSK-3β IC_{50} (nM)	Aurora A IC_{50} (nM)
20a	Ph	Ph	52%[a]	2800	ND
20b	Ph	Ph	330	628	ND
10	Ph	4-$PhCO_2H$	267	146	346
21b	Ph	4-$PhCO_2H$	32	49	292
22b	Ph	4-$Ph(CH_2)_2CO_2H$	20	24	375
23a	Ph	4-*trans*-*c*Hex-OH	172	281	ND
23b	Ph	4-*trans*-*c*Hex-OH	123	279	25%[a]
24b	Ph	4-$PhCH{=}CHCO_2H$	13	7	112
25a	Ph	4-$PhOCH_2CO_2H$	74	92	85
25b	Ph	4-$PhOCH_2CO_2H$	9	13	128
26a	Ph	4-$PhOCH_2CO_2Me$	ND	41%[a]	ND
26b	Ph	4-$PhOCH_2CO_2Me$	204	477	ND
27b	Ph	Isobenzofuran-[3H]-1-one	23	23	7300
28a	Adamantyl	4-$PhCO_2H$	34	26	ND
28b	Adamantyl	4-$PhCO_2H$	80	75	910
29a	3-OH-adamantyl	4-$PhCO_2H$	4.5	3	156
29b	3-OH-adamantyl	4-$PhCO_2H$	66	51	ND
30a	Adamantyl	4-$Ph(CH_2)_2CO_2H$	38	105	ND
31b	3-OH-adamantyl	4-$PhOCH_2CO_2H$	96	169	ND
32a	3-OH-adamantyl	4-*trans*-*c*Hex-OH	123	198	33%[a]
32b	3-OH-adamantyl	4-*trans*-*c*Hex-OH	444	744	23%[a]

ND, IC_{50} not determined.
[a] % Inhibition at 10 μM.

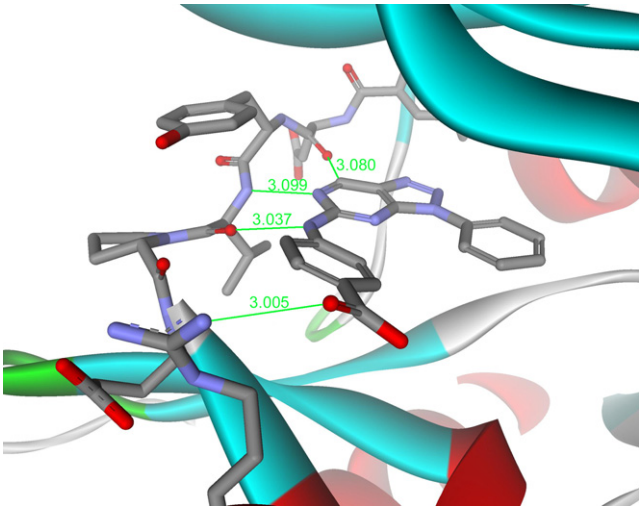
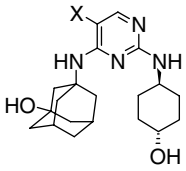


Figure 1. Docking model of compound **20b** into GSK-3β X-ray crystal structure 1q5k using Glide (Schrödinger) showing hydrogen bonds to the hinge region and to Arg-141 of GSK-3β.

occupied by the ATP ribose group and that the phenyl group was only partially filling this pocket. The adamantyl group is approximately the size of a phenyl rotated through 180° and was envisioned as replacement for the phenyl group to increase lipophilicity (potentially improving BBB penetration) and gain van der Waals binding energy. Nitropyrimidines with this moiety (**28a**) were more potent than similarly substituted azapurines (**28b**), probably because some flexibility was required to allow the adamantyl group to access the proper pocket. The 3-hydroxyadamantyl in **29a** improved potency to single-digit nanomolar, albeit with modest Aurora A selectivity. However, by replacing the carboxyphenyl group with *trans*-4-hydroxycyclohexyl, we were able to maintain 100 nM activity with good Aurora A selectivity.

Table 5
GSK-3α and GSK-3β assay results for compounds **33–35**



Compound #	X	GSK3α IC_{50} (nM)	GSK3β IC_{50} (nM)	Aurora A % inh, 10 μM
33	CF_3	61	41	45%
34	Br	91	125	69%
35	Cl	133	230	65%
36	F	1499	ND	ND

ND, IC_{50} not determined.

We also found that the nitro group was no longer essential and that other substitutions (Table 5, compounds **33** and **34**) actually offered improvements to sub 100 nM potency. This was approximately 10-fold improved potency compared to the phenyl analogs **14** and **11**, respectively. This suggested that the binding energy of the hydroxyadamantyl group was more important for potency than the hydrogen bond to Asp-133. In vivo studies of compound **33** in rat (Table 6) showed it to have 34% oral bioavailability and good exposure. Clearance and half-life were consistent with once-daily dosing, however, the low volume of distribution remains a challenge for attaining an efficacious CNS drug. Compounds of this class are undergoing further studies which will be reported in due course.

Table 6
Pharmacokinetic parameters for compound **33**

Dose	C_{max} (μg/mL)	T_{max} (h)	AUC (μg h/mL)	CL (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	*F*%
iv (5 mg/kg)	24.2		20.1	4.2	0.3	3.7	
po (20 mg/kg)	5.7	0.5	27.4			6.1	34

References and notes

1. Doble, B. W.; Woodgett, J. R. *J. Cell. Sci.* **2003**, *116*(7), 1175.
2. Cohen, P.; Goedert, M. *Nat. Rev. Drug Disc.* **2004**, *3*, 479.
3. Phiel, C. J.; Wilson, C. A.; Lee, V. M.-Y.; Klein, P. S. *Nature* **2003**, *423*, 435.
4. Assays for GSK-3 and Aurora A kinase inhibition were determined using Z'lyte reagents (Invitrogen) and performed using standard conditions provided by the manufacturer.
5. Cardozo, M. G.; Cogan, D.; Cywin, C. L.; Dahmann, G.; Disalvo, D.; Ginn, J. D.; Prokopowicz, A. S.; Spero, D. M.; Young, E. R. R. PCT Int. Appl. **2004**, WO 2004067516 A1.
6. Biwersi, C. M.; Mcnamara, D. J.; Repine, J. T.; Toogood, P. L.; Vanderwel, S. N.; Warmus, J. S. PCT Int. Appl. **2004**, WO 2004065378 A1.
7. Bryant, J.; Kochanny, M.; Yuan, S.; Khim, S.-K.; Buckman, B.; Arnaiz, D.; Boemer, U.; Briem, H.; Esperling, P.; Huwe, C.; Kuhnke, J.; Schaefer, M.; Wortmann, L.; Kosemund, D.; Eckle, E.; Feldman, R.; Phillips, G. *PCT Int. Appl.* **2004**, WO 2004048343 A1.
8. Dahmann, G.; Himmelsbach, F.; Wittneben, H.; Pautsch, A.; Prokopowicz, A. S.; Krist, B.; Schnapp, G.; Steegmaier, M.; Lenter, M.; Schoop, A.; Steurer, S.; Spevak, W. *PCT Int. Appl.* **2003**, WO 2003032997 A1.
9. Ustynyuk, Yu. A.; Subbotin, O. A.; Buchneva, L. M.; Kazitsyna, L. A. *Doklady Akademii Nauk SSSR* **1976**, *227*(1), 101.
10. Bhat, R.; Xue, Y.; Berg, S.; Hellberg, S.; Ormo, M.; Nilsson, Y.; Radesater, A. C.; Jerning, E.; Markgren, P. O.; Borgegard, T.; Nylof, M.; Gimenez-Cassina, A.; Hernandez, F.; Lucas, J. J.; Diaz-Nido, J.; Avila, J. *J. Biol. Chem.* **2003**, *278*, 45937.
11. Pierce, A. C.; Sandretto, K. L.; Bemis, G. W. *Proteins* **2002**, *49*, 567.
12. Ludovici, D. W.; Connors, R. W.; Coats, S. J.; Liu, L.; De Corte, B. L.; Johnson, D. L.; Schulz, M. J. PCT Int. Appl. **2006**, WO 2006076442 A2.
13. Freyne, E. J. E.; Lacrampe, J. F. A.; Perera, T. P. S.; Ten Holte, P.; Ligny, Y. A. E.; Lardeau, D. Y. R.; Lavrijssen, T. *PCT Int. Appl.* **2006**, WO 2006074985 A1.
14. Freyne, E. J. E.; Love, C. J.; Cooymans, L. P.; Vandermaesen, N.; Buijnsters, P. J. J. A.; Willems, M.; Embrechts, W. C. J. PCT Int. Appl. **2005**, WO 2005012307 A1.
15. Freyne, E. J. E.; Love, C. J.; Cooymans, L. P.; Vandermaesen, N.; Buijnsters, P. J. J. A.; Willems, M.; Embrechts, W. C. J. PCT Int. Appl. **2005**, WO 2005012304 A2.