Bioorganic & Medicinal Chemistry Letters 20 (2010) 1661–1664

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

3-Aryl-4-(arylhydrazono)-1*H*-pyrazol-5-ones: Highly ligand efficient and potent inhibitors of GSK3β

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ARTICLE INFO

Article history:
Received 16 December 2009
Revised 7 January 2010
Accepted 11 January 2010
Available online 21 January 2010

Keywords:
GSK3β
Kinase inhibitors
Pyrazolones
Ligand efficiency
Binding efficiency
Hit-to-lead
Lead-like

ABSTRACT

A series of 3-aryl-4-(arylhydrazono)-1*H*-pyrazol-5-one inhibitors of GSK3β was developed from a low molecular weight, highly ligand efficient screening hit **1**. Hit-to-lead optimization led to a number of highly potent inhibitors, while maintaining the high ligand efficiency of the screening hit.

Glycogen synthase kinase (GSK) is a Ser/Thr kinase, originally identified as the enzyme responsible for the inactivating phosphorylation of glycogen synthase.[1] Two isoforms, GSK3α (51 kDa) and GSK3β (47 kDa) are known and there is high homology in their kinase domains. The functional differences between the isoforms are currently unclear, but they are differently distributed[2] and not entirely functionally redundant, as GSK3β knockout in mice was shown to be embryonically lethal.[3] Subsequent research has identified GSK3 as a key kinase in a variety of signalling pathways and networks, and a potential therapeutic target for a number of diseases,[4,5] including bipolar disorder,[6,7] schizophrenia,[7] Alzheimer's disease,[8–10] cardiac disease,[11] and diabetes.[12,13] Much of the complexity of the GSK3 signalling network remains to be unravelled and small molecule intervention has begun to shed light on the overriding pharmacological action of GSK3 modulation. A number of GSK3 inhibitors have now entered clinical trials[14] the results of which will help define the ultimate practical utility of GSK3 inhibition.

In the course of screening our compound collection for inhibitors of GSK3β, we identified compound **1**, (*Z*)-4-(2-(2-chlorophenyl)hydrazono)-1*H*-pyrazol-5(4*H*)-one, as a low molecular weight inhibitor (MW = 222.6; K_i = 1.49 μM: Figure 1). This compound was notable amongst the screening hits in displaying an unusually high ligand efficiency. Various researchers have proposed the concepts of ligand,[15] or binding[16] efficiency as measures of the average value of the contributions of each atom within the binding molecule to the overall energy of binding to the target. We have chosen to use the binding efficiency index (BEI), which is simply calculated from activity and molecular weight,[16] to provide guidance in optimizing potency. Thus, BEI = (pK_i/MW), where molecular weight is in kiloDaltons. For compound **1**, BEI = 26.2, which was the second highest value that we recorded in this screen and amongst the highest we have observed in any kinase screen. With this starting point we believed that highly potent GSK3β inhibitors with properties suitable for lead optimization could be identified.[17]

In our initial binding model of compound **1** we hypothesized that the 4-hydrazino group was most likely forming an internal hydrogen bond with the pyrazolone carbonyl forming a pseudobicyclic 6,5-ring system, with a single substituent, the 2-chlorophenyl group. In this model, the pyrazolone heteroatoms would likely form two hydrogen bonds with the kinase hinge region, similar to those formed between GSK3 and a number of inhibitors, such as indirubin and derivatives (e.g., BIO),[18] maleimides (e.g., SB-415286),[19] and staurosporine[20] (Fig. 1). In this model, the 2-chlorophenyl ring of **1** occupies space that is also, in part, occupied by these inhibitors, while the pyrazolone 3-position is open and presents a suitable vector for additional interaction with the enzyme active site.

To explore and develop structure–activity relationships of the pyrazolones, a limited series of analogs was prepared by the general method shown in Scheme 1.

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doi:10.1016/j.bmcl.2010.01.072

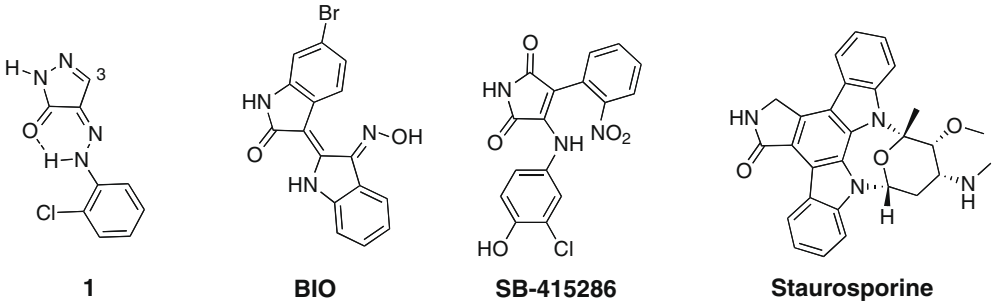
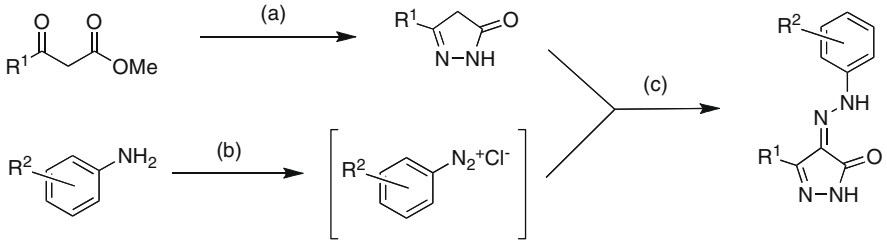


Figure 1. Compound **1** and structurally related inhibitors of GSK3β.

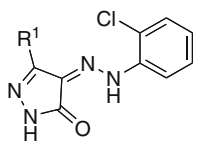


Scheme 1. Synthesis of pyrazolones. Reagents and conditions: (a) NH_2NH_2, EtOH; (b) $NaNO_2$, HCl, H_2O, 0 °C; (c) NaOAc, EtOH, H_2O.

In Scheme 1, ketoesters (prepared as needed from methylketones by the method of Jung et al.[21]) were converted to pyrazolones in good yield by treatment with hydrazine in ethanol. Subsequent treatment with freshly prepared aryl diazonium salts afforded the desired products, which were purified by HPLC.[22] In an initial evaluation of the screening hit we prepared a number of analogs from available ketoesters and the 2-chlorophenyl diazonium salt. Results of this series are summarized in Table 1.

Results from this survey showed that, in general, the pyrazolone 3-substituent had little effect on the inhibitory potency of the molecules, with the exception of compound **9**, which was 30-fold more potent than the screening hit **1**. Interestingly, this marked improvement in potency for compound **9** does not translate into a binding efficiency comparable to compound **1**. In fact, compound **9** is comparable in binding efficiency to the parent phenyl derivative, **4**. A careful analysis of the binding modes of these compounds suggested that their binding affinity might be limited by the presence of the *ortho*-chloro substituent. Glide docking[24] and subsequent minimization of compound **1** into the crystal structure of GSK3β complexed with staurosporine (Pdb code 1Q3D) gives two possible binding modes shown in Figure 2. The ligand conformation shown in Figure 2A places the chlorine unfavourably close to the carbonyl of Val135. In the alternate binding mode shown in Figure 2B, the *o*-chlorophenyl ring is rotated approximately 180°, so that there is no steric clash with the protein, but the intramolecular contact between the chlorine atom and the hydrazone is disfavoured. To address this liability and further explore the SAR of the series, we prepared a more diverse set of pyrazolone derivatives, with variation at R^1 and R^2. Data is shown in Table 2.

Table 1
3-Substituted 4-(2-(2-chlorophenyl)hydrazono)-1*H*-pyrazol-5(4*H*)-one inhibitors of GSK3β



Compd	R^1	MW	GSK3β, K_i, nM[23]	BEI
1	H	222.6	1490	26.2
2	Me	236.7	460	26.8
3	*i*Pr	264.7	>3700	<20.5
4	Ph	298.7	720	20.6
5	2-Pyridyl	299.7	650	20.6
6	3-Pyridyl	299.7	200	22.4
7	4-Pyridyl	299.7	250	22.0
8	3-MeO-Ph	328.8	850	18.5
9	3,4-$(MeO)_2$-Ph	358.8	44	20.5

It is apparent from the data contained in Table 2 that this scaffold is particularly suited to very potent inhibition of the target GSK3β. Numerous analogs possess low nanomolar and sub-nanomolar inhibition constants. With R^1 fixed as phenyl, 3- and 4-substitution at R^2 afforded compounds that were both potent and efficient inhibitors (e.g., **12**, **14**, and **15**). Fixing R^2 as 4-methoxyphenyl (**16**–**23**) identified R^1 as 3-pyridyl (**17**), as well as 3-methoxyphenyl (**19**), 4-methoxyphenyl (**20**) and 3,4-dimethoxyphenyl (**21**) as potent and efficient inhibitors. The additional methoxy group of the 3,4,5-trimethoxyphenyl-substituted compound **22**, though quite potent, was notably less ligand efficient. The R^1 combination of 3-pyridyl with 4-methoxy in compound **23** was potent (2 nM) and more efficient than **1**. Further optimization of this class of GSK3β inhibitors is exemplified by compounds **24** to **62**. As noted above, it does not necessarily follow that the most potent inhibitors are the most efficient inhibitors. Compounds **33**, **43**, **52**, **53**, and **62**, are all sub-10 nM GSK3β inhibitors, yet are significantly less efficient (BEI <22) than the screening hit, **1**. These compounds all bear 4-amino derivatives that would be expected to extend into solvated space. While the morpholino group might improve the solubility of these molecules, there is a significant efficiency cost of introducing a 100 Da substituent that contributes little, or anything, to the binding energy. Meanwhile, compounds **37**, **39**, and **54** are not only potent inhibitors (GSK3β K_i <5 nM), but are significantly improved in ligand efficiency (BEI >28) relative to **1**. Selectivity for these compounds against a panel of kinases (including kinases, such as CDK2, known to bind GSK3 inhibitors[25] and a variety of other Ser/Thr, Tyr and lipid kinases) is

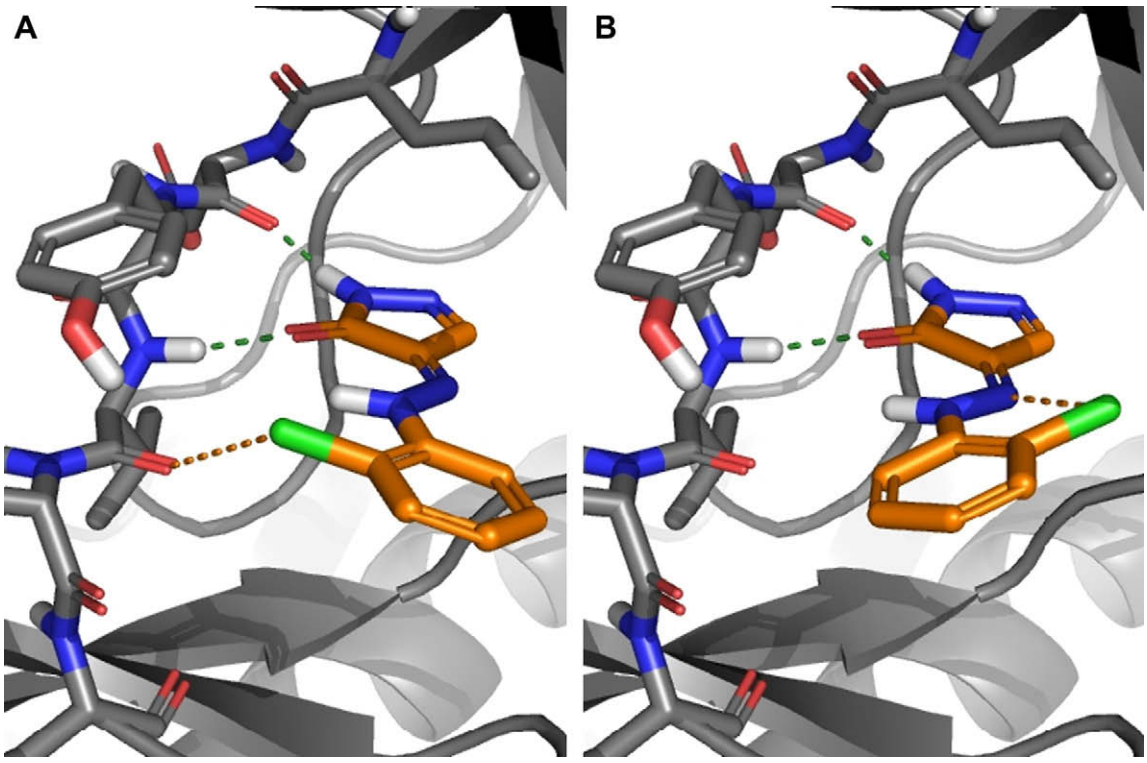
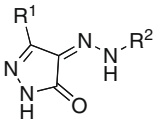


Figure 2. Binding model of compound **1** docked in GSK3β (PDB ID: 1q3d).[20]

Table 2
N-, 3-Substituted 4-(2-hydrazono)-1*H*-pyrazol-5(4*H*)-one inhibitors of GSK3β

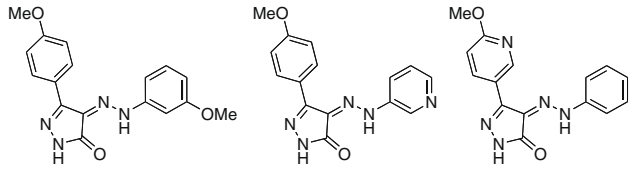


Compd	R^1	R^2	MW	GSK3b, K_i, nM[12]	BEI
10	Ph	Ph	264.3	99	26.5
11	Ph	3-Cl-Ph	298.7	120	23.2
12	Ph	4-Cl-Ph	298.7	23	25.6
13	Ph	2-MeO-Ph	294.3	>4000	<18.3
14	Ph	3-MeO-Ph	294.3	35	25.3
15	Ph	4-MeO-Ph	294.3	20	26.2
16	2-Pyridyl	4-MeO-Ph	295.3	640	21.0
17	3-Pyridyl	4-MeO-Ph	295.3	20	26.1
18	4-Pyridyl	4-MeO-Ph	295.3	30	25.5
19	3-MeO-Ph	4-MeO-Ph	324.3	18	23.9
20	4-MeO-Ph	4-MeO-Ph	324.3	2	26.8
21	3,4-$(MeO)_2$-Ph	4-MeO-Ph	354.4	<2	>24.5
22	3,4,5-$(MeO)_3$-Ph	4-MeO-Ph	384.4	9	20.9
23	4-MeO-3-pyridyl	4-MeO-Ph	325.3	2	26.7
24	3-MeO-Ph	Ph	294.3	39	25.2
25	3-MeO-Ph	3-Cl-Ph	328.8	8	24.6
26	3-MeO-Ph	4-Cl-Ph	328.8	9	24.5
27	3-MeO-Ph	3-MeO-Ph	324.3	5	25.6
28	3-MeO-Ph	4-CN-Ph	319.3	4	26.3
29	3-MeO-Ph	2-Pyridyl	295.3	8	27.4
30	3-MeO-Ph	3-Pyridyl	295.3	30	25.5
31	3-MeO-Ph	4-Pyridyl	295.3	23	25.9
32	3-MeO-Ph	4-CO_2H-Ph	338.3	6.5	24.2
33	3-MeO-Ph	4-NMe_2-Ph	337.8	9	23.8
34	3-MeO-Ph	4-Morpholino-Ph	379.4	59	19.1
35	4-MeO-Ph	3-Cl-Ph	328.8	1.9	26.5
36	4-MeO-Ph	4-Cl-Ph	328.8	2	26.5

Table 2 (*continued*)

Compd	R^1	R^2	MW	GSK3b, K_i, nM[12]	BEI
37	4-MeO-Ph	3-MeO-Ph	324.3	0.8	28.0
38	4-MeO-Ph	4-CN-Ph	319.3	<2	>27.2
39	4-MeO-Ph	3-Pyridyl	295.3	2	29.5
40	4-MeO-Ph	4-Pyridyl	295.3	14	26.6
41	4-MeO-Ph	4-NMe_2-Ph	337.4	110	20.6
42	4-MeO-Ph	4-NEt_2-Ph	365.3	16	21.3
43	4-MeO-Ph	4-Morpholino-Ph	379.4	8	21.3
44	3,4-$(MeO)_2$-Ph	Ph	324.3	<3.5	>26.1
45	3,4-$(MeO)_2$-Ph	3-Cl-Ph	358.8	0.4	26.2
46	3,4-$(MeO)_2$-Ph	4-Cl-Ph	358.8	<2	>24.2
47	3,4-$(MeO)_2$-Ph	3-MeO-Ph	354.4	0.4	26.5
48	3,4-$(MeO)_2$-Ph	4-CN-Ph	349.3	<2	>24.9
49	3,4-$(MeO)_2$-Ph	3-Pyridyl	325.3	<3.5	>26.0
50	3,4-$(MeO)_2$-Ph	4-Pyridyl	325.3	<3.5	>26.0
51	3,4-$(MeO)_2$-Ph	4-NMe_2-Ph	367.4	87	19.2
52	3,4-$(MeO)_2$-Ph	4-NEt_2-Ph	395.5	2	22.0
53	3,4-$(MeO)_2$-Ph	4-Morpholino-Ph	409.4	3	20.8
54	4-MeO-3-pyridyl	Ph	295.3	4.5	28.3
55	4-MeO-3-pyridyl	4-Cl-Ph	329.7	3	25.8
56	4-MeO-3-pyridyl	2-MeO-Ph	325.3	270	20.2
57	4-MeO-3-pyridyl	3-MeO-Ph	325.3	3.7	25.9
58	4-MeO-3-pyridyl	4-CN-Ph	320.3	8.3	25.2
59	4-MeO-3-pyridyl	3-pyridyl	296.3	23	25.8
60	4-MeO-3-pyridyl	4-Pyridyl	296.3	16	26.3
61	4-MeO-3-pyridyl	4-CO_2H-Ph	339.3	7.7	23.9
62	4-MeO-3-pyridyl	4-Morpholino-Ph	380.4	50	19.1

Table 3
Kinase selectivity profile of compounds **37**, **39** and **54**



37 **39** **54**

Kinase	**37** K_i (nM)	**39** K_i (nM)	**54** K_i (nM)
GSK3b	0.8	2	4.5
CDK2	95	62	76
Flt3	66	130	290
IRAK4	690	1500	1100
JAK2	>1600	>4000	3500
JNK3	>4000	>4000	2500
PI3Kg	1300	1000	540
KDR	1800	3400	1000
MET	>4000	>4000	>4000
PKA	>4000	>4000	>4000
Plk1	>4000	>4000	>4000
ROCK1	>4000	>4000	>4000
Src	>4000	>4000	>4000
Syk	690	>4000	1800

shown in Table 3 and shows good overall selectivity (minimally >20×) for an early lead series.[25]

During the course of this work we were able to obtain the X-ray crystallographic structure[26] of compound **46** (GSK3β K_i <2 nM; BEI >24.2) bound to GSK3β, which confirms the expected binding mode (Fig. 3). In this structure, hydrogen-bonding contacts are made between the pyrazolone NH and CO and the backbone of hinge residues Asp133 and Val135, respectively. In addition, the methoxy substituents (R^1 = 3,4-dimethoxyphenyl) make hydrogen-bonding contacts with both the backbone NH of Asp200 and the sidechain amine of Lys85. It appears that this set of hydrogen bonds, plus the hydrophobic contacts made by the two phenyl rings of the scaffold, form a maximally efficient binding core in GSK3. Ligands lacking any of these elements are generally less potent, though they may be equally efficient, while molecules containing additional functionality fall off in binding efficiency, though they may be equally potent.

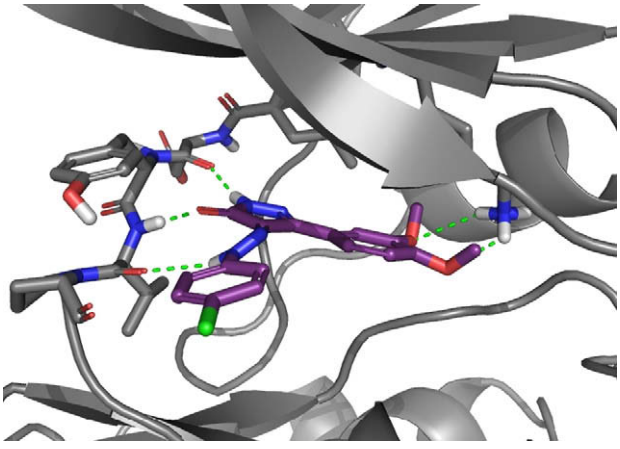


Figure 3. X-ray crystal structure of compound **46** bound to GSK3β (PDB ID: 3L1S).

By employing the simple binding efficiency index calculation to a screening hit, it has been possible to optimize a new class of GSK3β inhibitors from 1.5 μM to <1 nM, without compromising binding efficiency. In doing so, molecular weights of the most potent and efficient molecules have been controlled and lie well within the range of lead-like molecules,[17] allowing for further optimization of properties necessary for an orally available, efficacious therapeutic molecule.

Acknowledgements

The authors thank Ann Grippo, Yu-Ping Luong, Christine Memmott, Cameron Stuver Moody and Paul Taslimi, for enzymology support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.072.

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22. Compounds described were prepared according to the following general procedure: To a solution of aniline (1.1 equiv) in 6 M HCl (2 mL/mmol) at 0 °C is added $NaNO_2$ (1.1 equiv) and the mixture stirred at 0 °C for 30 min. The solution is added to the pyrazolone (1 equiv in 50% aqueous EtOH, 3 mL/mmol) and potassium acetate (6 equiv) and the mixture stirred at 0 °C for a further 30 min. The precipitate is filtered, washed with water and dried. Products are purified by HPLC.
23. Compounds were assayed by modification the coupled assay method described in: Fox, T.; Coll, J. T.; Xie, X.; Ford, P. J.; Germann, U. A.; Porter, M. D.; Pazhanisamy, S.; Fleming, M. A.; Galullo, V.; Su, M. S.; Wilson, K. P. *Protein Sci.* **1998**, *7*, 2249. Assay details are provided in the Supplementary data.
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26. Crystallographic data for the compound **46** bound to GSK3b has been deposited with the RCSB Protein Data Bank. PDB ID: 3L1S.