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Bioorganic & Medicinal Chemistry Letters 16 (2006) 2091–2094
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation of novel heterocyclic inhibitors of GSK-3

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Received 16 December 2005; revised 16 January 2006; accepted 17 January 2006
Available online 7 February 2006

Abstract—A set of novel heterocyclic pyrimidyl hydrazones has been synthesized as inhibitors of glycogen synthase kinase-3 (GSK-3) with the most active exhibiting low nanomolar activity. Quantum mechanical calculations indicate that of the conformational factors that could determine binding affinity, the planarity of the phenyl ring in relation to the central core and the conformation of the hydrazone chain may be the most influential.
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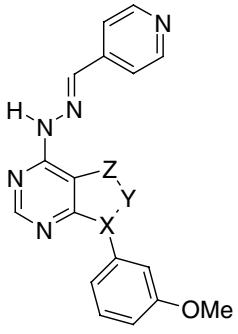
Glycogen synthase kinase-3 (GSK-3), a protein in the serine/threonine kinase family, is broadly expressed and serves many functions within the human body.[1] Among some of the diseases that GSK-3 may affect are Alzheimer's disease, diabetes, various cancer types, and neurological disorders.[2] One function of GSK-3 is to mediate the conversion of glycogen to glucose and is regulated in part by insulin signaling. In patients with insulin resistance, GSK-3 is constituently active, which leads to an increase in plasma glucose levels and hyperglycemia.[3] Inhibitors of GSK-3 could reduce glucose levels by mimicking the effect of insulin signaling on GSK-3 and thus could be used as anti-diabetic treatments. As part of our research efforts to discover effective medicines for the treatment of metabolic diseases, we became interested in inhibitors of GSK-3 as a potential treatment for diabetes.

We recently disclosed a series of GSK-3 inhibitors designed around a pyrazolopyrimidine template (**1**, Fig. 1).[4] While the compounds described therein were interesting inhibitors that showed a broad spectrum of activity against GSK-3, our efforts were mainly focused on peripheral modifications but retained a pyrazolopyrimidine core. This communication describes our efforts to discover potent GSK-3 inhibitors in which the pyrazole portion of the core structure was varied, as exemplified by structures **2**–**6** (Fig. 1).[5]



1 X=N, Y=N, Z=CH
2 X=C, Y=CH, Z=NH
3 X=C, Y=CH, Z=NMe
4 X=N, Y=CH, Z=CH
5 X=N, Y=CH, Z=N
6 X=C, Y=N, Z=NH

Figure 1.

The binding of **1** to the ATP-binding pocket of GSK-3 has been described and several key interactions were observed (Fig. 2).[6] Hydrogen bonding interactions of **1** with the β-strand of GSK-3 are required for binding, and for this reason we opted to retain the hydrazinopyrimidine portion of the core. The hydrazone moiety was shown to adopt an S-*cis* conformation which provides a desirable binding interaction with the protein backbone.[4] Previous structure–activity relationships (SAR) suggested that the phenyl ring at N-1 is required to exist in a co-planar arrangement with the pyrazolopyrimidine core in order to access a narrow pocket of the protein. We became interested in pyrazole modifications as a way to further enhance the co-planar relationship of

Keywords: GSK-3; Heterocycles; Diabetes; Inhibitors.
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doi:10.1016/j.bmcl.2006.01.057

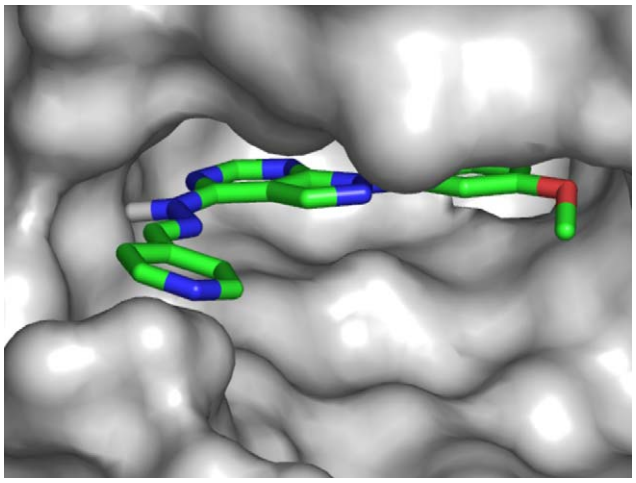


Figure 2. Model of **1** docked into the active site of GSK-3.[7] This representation depicts the S-*cis* conformation of the hydrazone and also indicates the importance of ring planarity of the 3-methoxyphenyl ring with the pyrazolopyrimidine core.

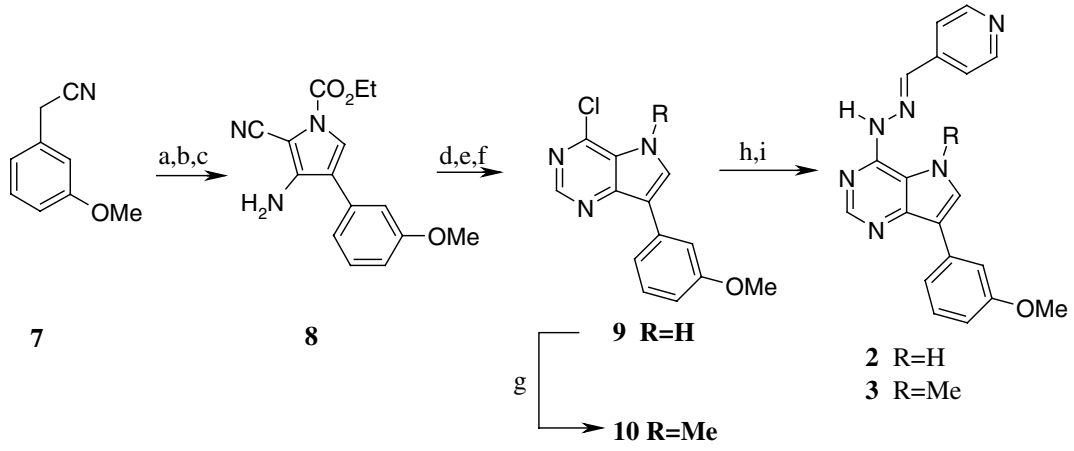
the phenyl ring with the core, as well as strengthen the S-*cis* conformation of the hydrazone.

The syntheses of the new inhibitors are described in Schemes 1–4.[4,5] The synthesis of **2** began using a modified literature procedure to access **8** (Scheme 1).[8] With the desired functionality present, **8** was treated with Na_2CO_3 to remove the carbamate protecting group on the pyrrole. Ring closure with formic acid and subsequent chlorination as previously described[4] provided the desired pyrrolopyrimidine **9**. The chloro group was displaced with hydrazine followed by condensation with pyridine-4-carboxaldehyde to provide **2**. Alternatively, the *N*-methyl derivative **3** could be accessed by methylation of **9**. Displacement of the chloro group and condensation as above gave **3**.

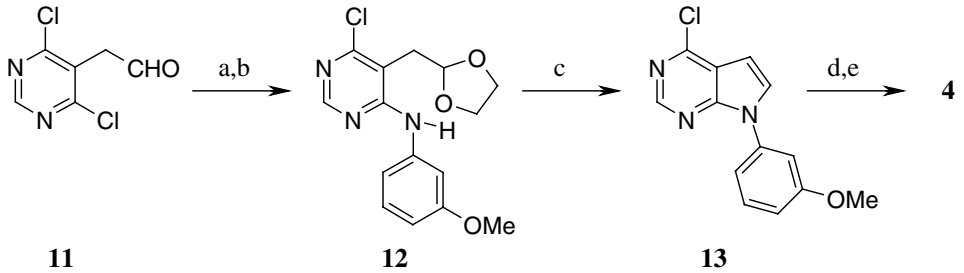
The preparation of pyrrolopyrimidine **4** is depicted in Scheme 2. Aldehyde **11**[9] was protected as the 1,3-dioxolane prior to chemoselective displacement of only one of the chloro groups to provide the pyrrole precursor **12**. Acid catalyzed hydrolysis of the 1,3-dioxolane and subsequent cyclization provided 4-chloropyrrolopyrimidine **13**. Compound **4** was obtained upon displacement of the chloro group with hydrazine hydrate and condensation with the appropriate aldehyde.

Imidazopyrimidine **5** was synthesized according to the outline depicted in Scheme 3. 5-Amino-4,6-dichloropyrimidine[10] was treated with 3-methoxyaniline to afford a monosubstituted product, which was subsequently treated with diethoxymethyl acetate[11] to afford ring closure and provide **15**. From that point the route described above was followed to install the hydrazone.

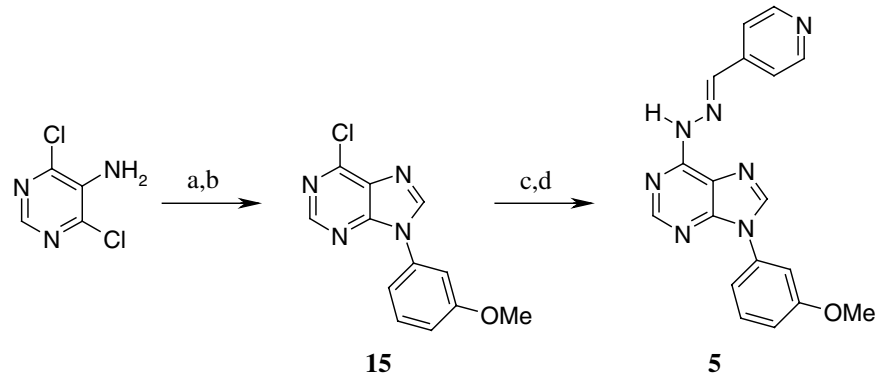
The synthesis of pyrazolopyrimidine **6** is shown in Scheme 4. (3-Methoxyphenyl)acetonitrile was treated with ethyl diazoacetate followed by ring closure with



Scheme 1. Reagents and conditions: (a) HCO_2Et, NaH; (b) H_2NCH_2CN, NaOAc, 80%; (c) ethyl chloroformate, DBN, 0 °C, then DBN, 0 °C to rt; (d) Na_2CO_3, MeOH, 71%; (e) HCO_2H, reflux, 75%; (f) $POCl_3$, 100 °C; (g) $(MeO)_2SO_2$, K_2CO_3, 75%; (h) hydrazine hydrate, EtOH, 80 °C, 82%; (i) pyridine-4-carboxaldehyde, pyrrolidine (cat.), EtOH, 80 °C, 80%.

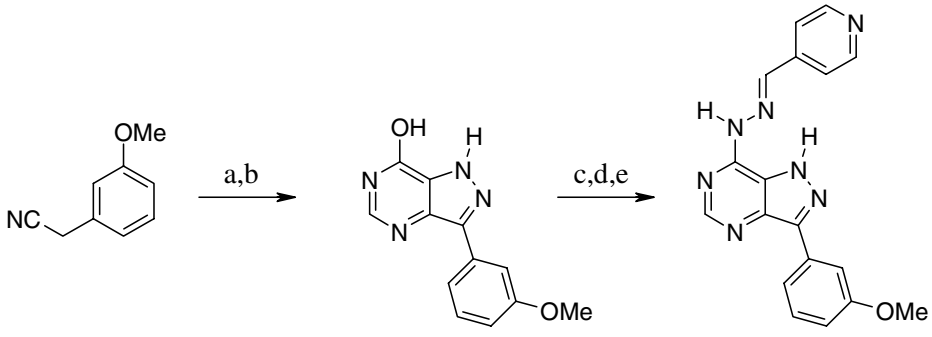


Scheme 2. Reagents and conditions: (a) ethylene glycol, *p*-TsOH, PhH, 100 °C, 64%; (b) *m*-anisidine, *p*-TsOH (cat.), THF, sealed tube, 130 °C, 39%; (c) 1.5 M aq HCl, THF, 80 °C, 61%; (d) hydrazine hydrate, EtOH, 80 °C, 44%; (e) pyridine-4-carboxaldehyde, pyrrolidine (cat.), EtOH, 80 °C, 78%.



15 5

Scheme 3. Reagents and conditions: (a) 3-methoxyaniline, EtOH, 98%; (b) diethoxymethyl acetate, Δ, 54%; (c) hydrazine hydrate, EtOH, 80 °C; (d) pyridine-4-carboxaldehyde, pyrrolidine (cat.), EtOH, 80 °C.



14 6

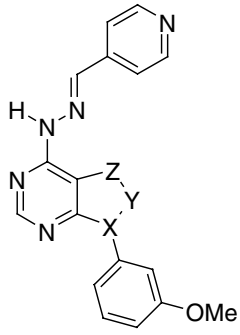
Scheme 4. Reagents and conditions: (a) EtO_2CCHN_2, NaOEt, NaOH, 29%; (b) formamidine acetate; (c) $POCl_3$; (d) hydrazine hydrate, EtOH, 80 °C; (e) pyridine-4-carboxaldehyde, pyrrolidine (cat.), EtOH, 80 °C.

formamidine acetate to provide **14**. Treatment with $POCl_3$ provided the chloro derivative, which was prosecuted in a similar manner as previously described to afford the desired product **6**.

Compounds **2**–**6** were evaluated as inhibitors of GSK-3 and the results are shown in Table 1.[12] The pyrrole analog **2** was equipotent to **1** (pIC_{50} = 8.3), however N-methylation (**3**) gave a 100-fold loss of activity. The isomeric pyrrole (**4**) was shown to be an order of magnitude less active than both **1** and **2**. Imidazole **5** was also significantly less active (pIC_{50} = 6.2), but the isomeric pyrazole (**6**) was shown to be equipotent with **1** and **2**. Thus, we observed that both **2** and **6** were comparable in activity to **1**.

While the discovery of potent core templates was exciting, we sought to explain the difference in activities of our inhibitors. We compared the conformations of **2**–**6** with that of **1** to determine the existence of any favorable binding interactions. One conformational aspect that had been previously observed was the preference for planarity of the phenyl ring at N-1.[4] The planarity can be modulated by *ortho* substituents on the phenyl ring, as these are in close proximity to N-2. One explanation of this effect is that with nitrogen at the 2-position (e.g, **1**), only a minimal steric interaction with the hydrogens on the *ortho*-position of the phenyl ring exists (Fig. 3). To assess the poten-

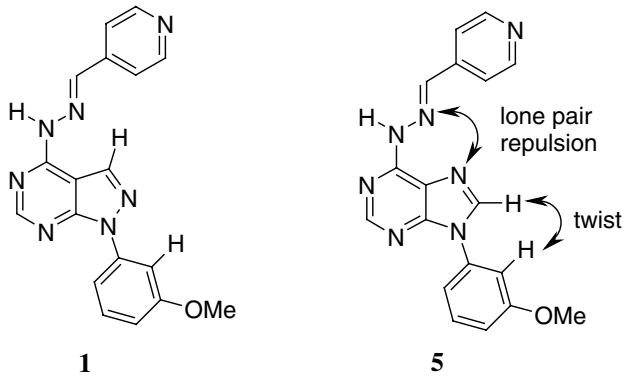
Table 1. Conformational energy and GSK-3 inhibitory activity of novel heterocyclic compounds **2**–**6**



Compound	X	Y	Z	pIC_{50}	*Cis* versus *trans* energy[a] (kcal/mol)	Planarity energy[b] (kcal/mol)
1	N	N	CH	8.2	−5.7	0.13
2	C	CH	NH	8.3	−8.3	0.61
3	C	CH	NMe	6.3	ND	ND
4	N	CH	CH	7.5	−4.7	3.2
5	N	CH	N	6.2	−0.06	2.6
6	CH	N	NH	8.3	−8.3	0.0

[a] The *cis* versus *trans* energy refers to the conformation of the hydrazone moiety.

[b] Planarity energy refers to the energy required to force a co-planar conformation between the phenyl ring and the core.



1 **5**

Figure 3.

tial importance of this interaction, ab initio calculations were performed on the inhibitors and are shown in Table 1.[13] The results suggest that increasing the twist of the phenyl ring could clearly account for the observed modulation in activity of our inhibitors. In addition, the conformation of the hydrazone chain is another factor influencing binding as seen in **2** and **6**. In both of these inhibitors, the S-*cis* hydrazone conformation is preferred due to an intramolecular hydrogen bond. Methylation of **2** removed the hydrogen bond (**3**), dramatically decreasing the activity.

When the substitution pattern is that as in **4**, the steric interactions of the phenyl and hydrogen at C-2 were amplified and the activity was reduced significantly. The trend was observed with **5** as well, as an order of magnitude decrease in binding was observed. However, this trend was not followed with pyrrole **2** which also contains a C–H at the 2-position. We believe that the increased bond length between C-1 and the phenyl ring in **2** lessens the non-covalent interaction between C-2 and the *ortho* hydrogens on the phenyl ring, thus reducing any potential contributions to the activity. In addition to the twist of the phenyl ring in compound **5**, N-3 interacts unfavorably with the nitrogen of the hydrazone (Fig. 3). This interaction could lower the preference of *cis* versus *trans* orientation, which in turn could be partially accountable for the observed decrease in activity.

In conclusion, we have synthesized novel heterocyclic inhibitors of GSK-3 based on a template previously discovered. The activity can be rationalized by computational analysis and thus can be modulated based on the heterocycle and the substitution pattern about the ring. The most active compounds were currently being further evaluated as potential anti-diabetic treatments and these results will be reported in due course.

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5. All novel compounds exhibited acceptable ^{1}H NMR and mass spectra, and were of >95% purity as determined by analytical HPLC.
6. Image was created with PyMol.
7. Structural coordinates for the GSK-3 structure were similar to those reported in: 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.
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12. GSK-3 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 μM peptide substrate (Biotin-Ahx-AAAKRREILSRRP-S(PO3)YR-amide), 2.5 μM ATP and 0.2 μCi/well [γ^{33}P]ATP. After 40 min, the reaction was stopped by addition of 100 mM EDTA and 1 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.
13. The energies were calculated at the HF/6-31G(d) level of theory with Gaussian 98 from Gaussian, Inc. The values reported reflect the energy difference of the *trans* hydrazone conformation relative to the *cis* hydrazone, with a value of 0 kcal/mol indicating that no preference between the two conformations exists. A positive number suggests that a preference for the *trans* hydrazone conformation exists and a negative number suggests that a preference for the *cis* hydrazone conformation exists.