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4-Acylamino-6-arylfuro[2,3-*d*]pyrimidines: potent and selective glycogen synthase kinase-3 inhibitors

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Abstract—Modeling studies of a furo[2,3-*d*]pyrimidine GSK-3 hit compound **1** superimposed onto the X-ray crystal structure of a legacy pyrazolo[3,4-*c*]pyridazine GSK-3 inhibitor **2** led to the identification of 4-acylamino-6-arylfuro[2,3-*d*]pyrimidine template **3**. Synthesis of analogues based on template **3** has resulted in a number of potent and selective GSK-3β inhibitors. The most potent and selective compound was the *m*-pyridyl analogue **24**.

1. Introduction

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase involved in the regulations of many cell functions.[1] GSK-3 is implicated in type-2 diabetes because of its ability to phosphorylate glycogen synthase, which effectively inhibits the enzyme in catalyzing the synthesis of glycogen. Recent publications have also suggested that the β-isomer (GSK-3β) of its two isoforms (α and β) plays a pivotal role in the genesis of Alzheimer's disease (AD) and other dementia.[2,3] A selective inhibitor of GSK-3 potentially can be an effective agent for the treatment of type-2 diabetes and/or AD, as well as other neurodegenerative disorders. A number of GSK-3 inhibitors having different structural classes have been reported, and they showed varying degrees of efficacy and specificity.[4]

In the course of our exploratory research targeting the VEGFR2 and TIE2 tyrosine kinases,[5] furo[2,3-*d*]pyrimidine **1** was identified as a GSK-3 inhibitor having an IC_{50} value of 0.081 μM versus GSK-3β. Modeling studies in which **1** was superimposed onto the X-ray crystal structure of the complex of the potent pyrazolo[3,4-*c*]pyridazine GSK-3 inhibitor **2**[6] with GSK-3β suggested that both compounds bind in the enzyme with similar space requirements. The studies also suggested the existence of a unique binding interaction between **1** and the hinge region in the ATP binding site (Figs. 1 and 2).[7]

The outcome of the modeling studies prompted us to modify **1** with a view to increasing its affinity for GSK-3. This included replacement of the *p*-methoxy group by other groups, the acylation of the 4-amino group, and removal of the pyridyl moiety, which resulted in the creation of a new template **3** (Fig. 2).

We first investigated the structure–activity relationship (SAR) on the 4-*N*-acyl portion. Thus a set of analogues of **3** with varying *N*-acyl groups and with a constant 6-*p*-methoxyphenyl group was synthesized. Their kinase inhibitory activity is summarized in Table 1. The hexanoyl analogue **4** exhibited submicromolar inhibitory activity versus GSK-3β. The closely related methylthio analogue **5** was equipotent to **4**. On the other hand, the isobutyryl analogue **6** was approximately 4-fold more potent than **5**. This interesting result provided the impetus for the synthesis of cycloalkyl analogues **7**–**10**. Unfortunately, **7** and **9** exhibited only slight improvements in the potency (IC_{50} = ca. 0.3 μM) over that of **4** or **5**, and the cyclohexyl analogue **10** was much less potent. Surprisingly, cyclopentyl analogue **8** was 10-fold

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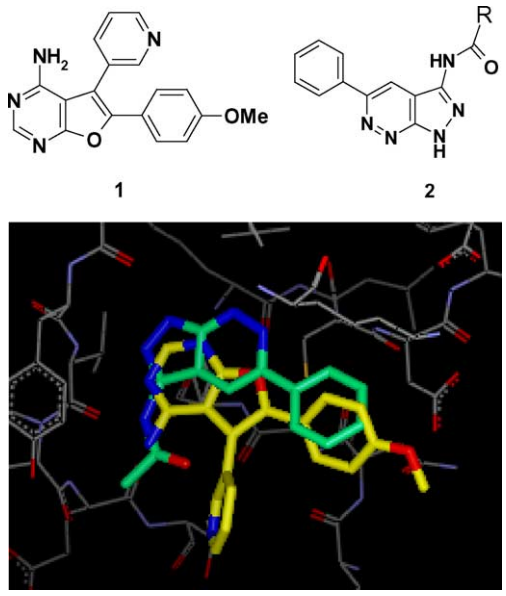


Figure 1. Docking study of furo[2,3-*d*]pyrimidine **1** (yellow) in an X-ray crystal structure of the complex of pyrazolo[3,4-*c*]pyridazine **2** (light green) and GSK-3β.

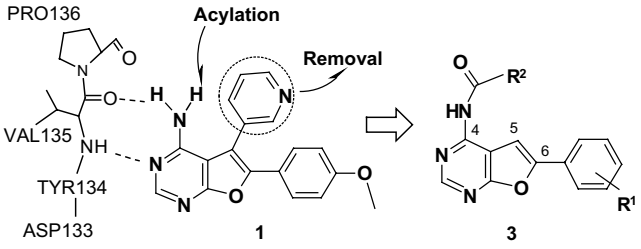


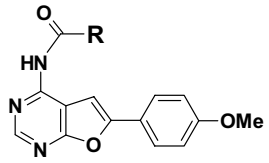
Figure 2. Proposed binding of a furo[2,3-*d*]pyrimidine hit **1** with the hinge region and design of a new series of GSK-3 inhibitor **3**.

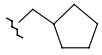
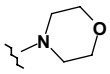
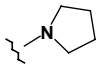
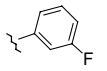
more potent than **7** or **9** and was by far the most potent analogue versus GSK-3. According to the modeling studies of analogue **8** (see Fig. 3), the methine hydrogen and vertical orientation of the cyclopentyl group appeared to have a unique binding interaction with PRO136 in the front hydrophobic pocket. To test this hypothesis, several compounds **11**–**14**, which were void of the methine hydrogen, were synthesized. These compounds ranged from morpholino and pyrrolidino analogues (**11** and **12**) having in essence a urea-type functional group to other aryl groups (**13** and **14**), and they exhibited weak GSK-3 inhibitory activity, maybe due to unfavorable affinity of the 4-*N*-acyl or urea substituent with PRO136. All the analogues **4**–**14** showed weak inhibitory activity against CDK-2 and VEGFR2.

In the ensuing SAR studies, we utilized cyclopropyl analogue **7** as the starting point and explored the modification of the 6-position of the furo[2,3-*d*]pyrimidine scaffold. The resultant analogues are listed in Table 2. As shown in the table, the unsubstituted **15** showed improved potency against GSK-3β. However, replacement with *p*-chloro, *p*-methyl, and *p*-fluoro groups (compounds **16**–**18**) gave analogues having reduced inhibitory activity, suggesting the potentially detrimental effects of a *p*-substituent.

Very interestingly, the most potent compound in the series was the *m*-pyridyl analogue **19**. Potency of **15**–**19** against VEGFR2 increased somewhat but those were still in the submicromolar to low micromolar range. These findings prompted us to prepare analogues **20**–**24** having identical 6-aryl groups as those in **15**–**19** but with

Table 1. Inhibition of hGSK-3β[8] by 4-*N*-acyl analogues **4**–**14**



No.	R	GSK-3β IC_{50}, μM	CDK2 IC_{50}, μM	VEGFR2 IC_{50}, μM
4	$-(CH_2)_4CH_3$	0.437	>19	>48
5	$-(CH_2)_2SCH_3$	0.537	>20	>47
6	$-CH(CH_3)_2$	0.132	>21	>48
7		0.316	>20	3.55
8		0.032	>19	1.02
9		0.324	>19	>48
10		1.82	>20	>48
11		>34	>20	>48
12		1.95	>21	12.3
13		>35	>21	21.9
14		2.40	>20	>48

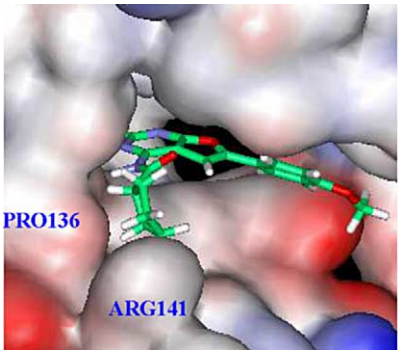
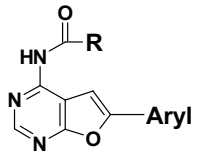
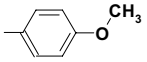
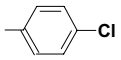
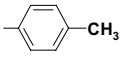
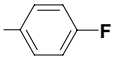
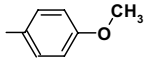
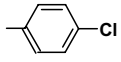
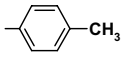
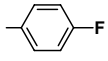


Figure 3. In silico surface model of the GSK-3 ATP-binding pocket with compound **8**.

Table 2. Inhibition of hGSK-3β[8] by 6-aryl analogues **7**, **8**, and **15**–**24**



No.	R	Aryl	GSK-3β IC_{50}, μM	CDK2 IC_{50}, μM	VEGFR2 IC_{50}, μM
7			0.316	>20	3.55
15			0.174	>21	0.832
16			1.00	>20	2.04
17			0.617	>21	0.631
18			0.575	>19	2.04
19			0.005	0.447	3.24
8			0.032	>19	1.02
20			0.023	>19	>23
21			0.550	>20	>20
22			0.240	>20	>20
23			0.219	>19	>19
24			0.005	0.457	>24

a cyclopentyl group in the 4-position. As a result, analogues **20**–**24** showed comparable potency versus GSK-3β to that of compounds **15**–**19**. However and importantly, these compounds showed a lack of inhibitory activity against CDK-2 and VEGFR2.

Having identified a series of potent GSK-3 inhibitors, we next profiled the most potent analogue **24** against a panel of more than 20 kinases, including GSK-3β (Table 3).[9] The data showed that analogue **24** indeed had excellent selectivity over all the kinases in the panel, including CDK-2. Overlaying of docking structures of **24** in GSK-3β and CDK-2/cyclin A[10] also suggested that the selectivity of **24** was likely due to a steric clash between a cyclopentyl group and its interacting site in CDK-2. Based on the modeling studies, however, *m*-pyridyl effect on the high potency was not clear. Finally, representative three analogues **19**, **20**, and **24** were examined in an assay that measured glycogen accumulation in L6 cells (Table 4). The 6-phenyl analogue **20** did not show potency in such an assay, but both *m*-pyridyl analogues **19** and **24** exhibited excellent potency of glycogen accumulation.[11]

2. Chemistry[12]

The furo[2,3-*d*]pyrimidine analogues were prepared according to procedures outlined in Scheme 1. The chemistry started with alkylation of commercially available 2-bromoacetophenones **25i**–**v** with malononitrile to give benzoylmethylmalononitriles **26i**–**v**, which were treated with hydrochloric acid in acetic acid at room temperature to afford 2-amino-3-cyanofurans **27i**–**v**. Cyclization of furan **27i**–**v** with formamide resulted in 4-amino-6-phenyl-furo[2,3-*d*]pyrimidine **28i**–**v**, which

Table 3. Selectivity of *m*-pyridyl analogue **24** for GSK-3β[a]

Compd no.	**24**
AMPK	0
Chk1	0
CKII	0
JNK	0
LCK	2
MAPK	5
RSK-2	NT
MAPKAP-K2	0
MEK1	0
MSK1	10
p70S6K	0
PDK1	0
PHOS.K	9
PKA	0
PKBα	1
PKCα	NT
PRAK	7
ROKα	0
SAPK2a	0
SAPK2b	0
SAPK3	0
SAPK4	17
SGK	8
CDK2/Cyclin A	66
GSK-3β	91

[a] Values are % *I* @ 10 μM using 100 μM ATP (see Ref. 9 for kinases used and assay details).

Table 4. Potency of **19**, **20**, and **24** inglycogen accumulation in L6 cells

No.	Potency of glycogen accumulation (EC_{50}, μM)
19	0.74
20	>10
24	0.39

were reacted with acylchlorides to afford the desired products. With regard to the synthesis of a *m*-pyridyl analogues **19** and **24**, the precursor **28vi** was obtained by a different method. Obtained 2-amino-3-cyano-furan **27vi** was converted into an ethoxyimino derivative, followed by treatment with ammonia in a solution of ethanol and tetrahydrofuran. Subsequently, cyclization was completed by addition of sodium ethoxide in the same solvent to give a precursor, 4-amino-6-(*m*-pyridyl)-furo[2,3-*d*]pyrimidine **28vi**. Cyclopentylcarbonylation of this precursor in a usual manner led to analogue **24**.

3. Conclusion

Modeling studies of a GSK-3β hit compound **1** by superimposing onto the X-ray crystal structure of the complex of **2** with GSK-3β led to the development of a template represented by structure **3**. Synthesis based on the template has led to the identification of a series of potent GSK-3β specific analogues. One such compound, the *m*-pyridyl analogue **24**, was found to exhibit potent GSK-3β inhibitory activity, possess excellent selectivity over other kinases including CDK-2 and VEGFR2, and exhibit excellent potency in cellular assays.

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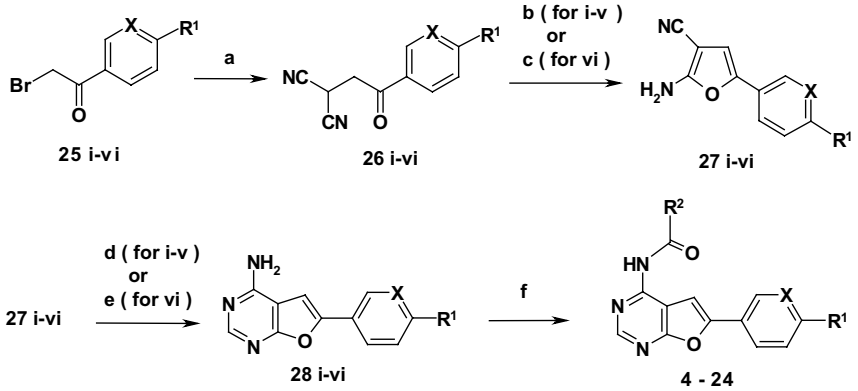

(i) X=CH, R^1=OCH_3; (ii) X=CH, R^1=H; (iii) X=CH, R^1=Cl; (iv) X=CH, R^1=CH_3; (v) X=CH, R^1=F; (vi) X=N, R^1=H

Scheme 1. Reagents and conditions: (a) malononitrile, NaOEt; (b) HCl–AcOH; (c) piperidine, reflux EtOH; (d) formamide, 200 °C; (e) 1. $(EtO)_2CHOAc$, 2. NH_3, EtOH–THF, 3. NaOEt, EtOH–THF; (f) acylchloride, NaH, DMF.

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