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Structure–activity relationship of the 7-hydroxy benzimidazole analogs as glycogen synthase kinase 3β inhibitor

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ABSTRACT

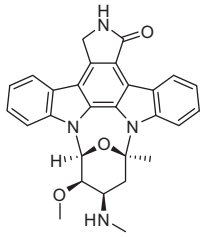
Design, synthesis and the GSK3β inhibitory activities of the 7-hydroxy benzimidazole analogs are described. The solid-phase synthetic route was also developed for preparation of the analogs consisting of the novel ATP competitive scaffold. In addition, the structure–activity relationship of the 7-hydroxy benzimidazole analogs and their biological activities are reported.

Glycogen synthase kinase 3 (GSK3) is a multi-functional serine/threonine kinase involved in many biological processes. GSK3α (51 kDa) and GSK3β (47 kDa) are known two isomers, which are highly homologous each other in their kinase domain. GSK3 controls the relevant substrates, such as glycogen synthase (GS), p53, p27, and β-catenin, which are associated with many diseases such as type II diabetes mellitus (T2DM), cancer, bipolar disorder, Alzheimer's disease, and schizophrenia.[1–3] GSK3 is a ubiquitous kinase that participates in a multitude of cellular processes, including cell membrane-to-nucleus signaling, gene transcription, translation, cytoskeletal structuring and cell cycle progression and survival. Thus, modulation of GSK3 has been considered as one of the promising therapeutic ways for the treatment of the corresponding human diseases.[4–6]

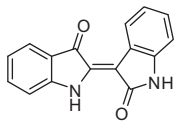
Up to date, a number of orthosteric GSK3β inhibitors have been proposed with a wide range of potencies at the discovery stage. In addition, the non-ATP competitive (allosteric) inhibitors in clinical trial have been reported in literature (Fig. 1).[7–11] We have recently reported 7-hydroxy benzimidazole scaffold as the novel ATP competitive GSK3β inhibitor through X-ray crystallography and docking studies.[12] The scaffold possesses an important hydroxyl group, which is involved in hydrogen-bonding with NH of Val135 and carbonyl of Asp133.

This implied that the 7-hydroxyl group functioned as a hydrogen bonding donor and as an acceptor.

Herein we described structure optimization of the 7-hydroxy benzimidazole scaffold, their biological evaluation and structure–activity relationship (SAR). The analogs were synthesized by the solid phase parallel synthetic method. The synthetic route to the modified 7-hydroxy benzimidazole is shown in Scheme 1. 3-Amino-4-methoxybenzoic acid **1** was converted into **2** via sequential esterification, imidation[13] and HCl-salt formation. Reaction of **2** with NaOCl followed by cyclization in the presence of Na_2CO_3 afforded the benzimidazole intermediate, which was further treated with $AlCl_3$ in toluene for the concomitant hydrolysis of ester and methoxy groups. Finally, the benzimidazole **3** was obtained by an esterification of the corresponding acid intermediate. The benzimidazole **3** was coupled with Wang resin[14] in the presence



Staurosporine



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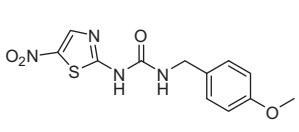

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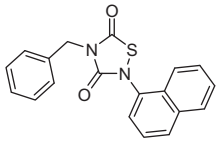

Tideglusib (NP-12)

Figure 1. Examples of the known GSK3 inhibitors.

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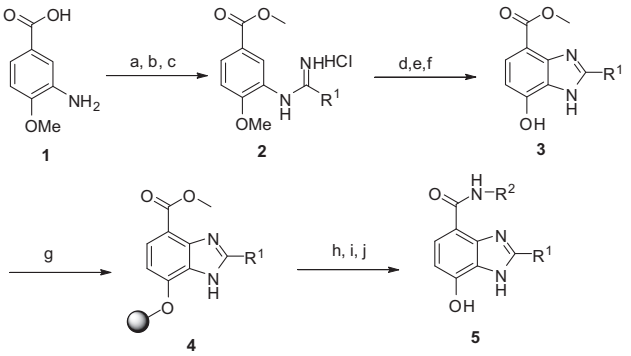


Scheme 1. Reagents and conditions: (a)MeOH, H_2SO_4, reflux, 90%; (b) R^1-CN, p-TSA, heat to 180–210 °C; (c) HCl (g), bubbling, rt; (d) NaOCl, $NaHCO_3$, 50% aq MeOH, rt 30–70% of three steps; (e) $AlCl_3$, toluene, reflux, 50–80%; (f) MeOH, H_2SO_4, reflux, 80–90%; (g) Wang bromo resin (black dot), Cs_2CO_3, KI, DMF, 60 °C; (h) LiOH·H_2O, MeOH, aq THF, rt, 90–95%; (i) R^2-NH_2, EDC, HOBt, DMAP, DMF, rt; (j) 10–50% TFA in DCM solution, rt, 20–80% of two steps.

of Cs_2CO_3 and KI in DMF at 60 °C. Hydrolysis of the ester **4** with LiOH and amidation of the resin supporting acid, in the presence of EDC, HOBt and DMAP, in DMF provided a variety of amide analogs. Finally, the solid resin was washed with MeOH, DMF and MC several times and then TFA treatment in DCM yielded the free 7-hydroxy benzimidazole analog **5**.

Initially, the synthetic route including solid phase chemistry was successfully developed for the facile derivatization of R^1 and R^2 of 7-hydroxy benzimidazole **5**. The linker for R^2 was fixed as amide, which has the optimal direction to the sugar and triphosphate binding site of ATP.

Our docking study (Fig. 2) revealed the oxygen of phenethyl sulfonamide moiety, which is linked to amide at the 4-position of the benzimidazole, would enhance the inhibitory activity by a hydrophilic interaction with Arg141 at the triphosphate and substrate binding region of the catalytic domain. Accordingly, the synthesis was commenced with preparation of the R^1 and R^2-diversified analogs by use of solid phase parallel reaction. The GSK3β kinase inhibitory activity was determined using the known method.[15] We first conducted the R^2-variation with R^1 fixed as a benzyl group. The GSK3β inhibitory activities were summarized in Table 1.

Initial SAR of the synthesized analogs revealed that introduction of the relatively small substituents for R^2 (**6** and **7**) provided the low inhibitory activities. However, introduction of the bulky aromatic moieties improved the inhibitory potency. In particular,

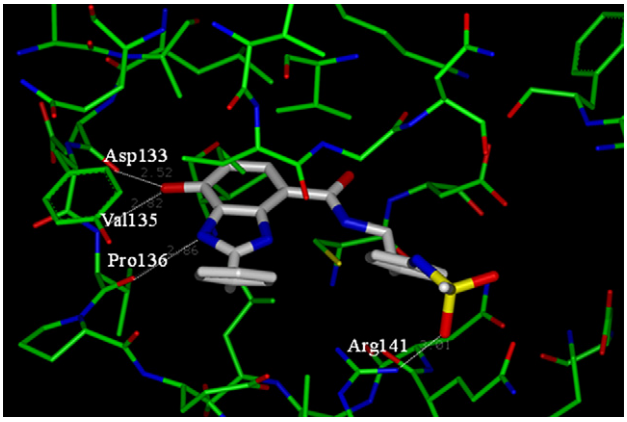
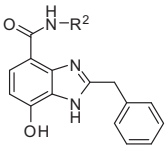
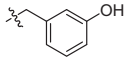
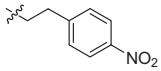
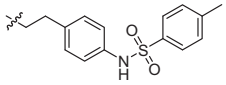
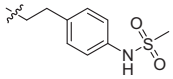


Figure 2. Binding mode of the analog **11** in the GSK3β binding pocket. Interaction of OH and NH of the hydroxy benzimidazole moiety with Asp133, Val135, and Pro136 with distances of 2.52, 2.82, and 2.86 Å are shown. The oxygen of the sulfonamide group also interacts with NH of Arg141 with distance of 3.01 Å.

Table 1
Inhibitory activities of the 7-hydroxy benzimidazole analogs against GSK3β



Analogs	R^2	Remaining activity at 5 μM (%)	IC_{50} (μM)
Ref. 16		~1	0.070
6	H	75	ND
7	Butyl	63	ND
8		53	ND
9		32	2.0
10		36	2.8
11		18	1.6

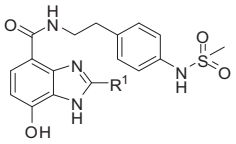
ND, IC_{50} not determined.

addition of the freely rotatable sp^3 carbon substituent to the aromatic moiety by amidation seemed to enhance the inhibitory activities of the analogs **8** and **9**. The analog **11** exhibited the best GSK3β inhibitory activity with IC_{50} of 1.6 μM. We turned our attention to the R^1-substituents effect on the inhibitory activity on the structural basis of **11**. The results were summarized in Table 2.

Interestingly, the analogs (**13**, **14**, **15**, **17**, **18** and **19**) directly linked to the cyclic R^1-substituents exhibited more potent GSK3β inhibitory activities compared to the analog **11**. In particular, the analogs **15** and **17** exhibited excellent inhibitory activities.

The cell based assay was also performed to confirm the intrinsic cell permeability and potency in cellular environment. The result of cellular assay revealed the similar tendency as shown in the enzyme assay as anticipated, although the analog **19** with an EC_{50} of 25 μM of cellular inhibitory activity showed an impermeable character. Other analogs directly linked to the substituted aromatic system (**15**, **16**, **17**, and **18**) showed good cell permeable character and the analog **15** exhibited the best potency in both enzymatic and cellular assays. Based on preliminary examination of the R^1

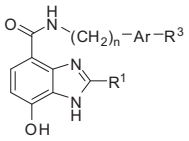
Table 2
Enzyme and cellular (Rd cells) inhibitory activities of the 7-hydroxy benzimidazole analogs against GSK3β



Analogs	R^1	IC_{50} (μM)	EC_{50} (μM)
11	Benzyl	1.600	3.90
12	Methyl	ND	21.00
13	Cyclopropyl	0.140	4.80
14	Cyclopentyl	0.044	2.70
15	4-Cl-phenyl	0.007	0.12
16	2,4-Di-F-phenyl	ND	0.58
17	4-F-phenyl	0.001	0.40
18	2-Cl, 4-F-phenyl	0.020	0.23
19	4-Pyridyl	0.110	25.00

ND, IC_{50} not determined.

Table 3
Enzymatic and cellular (Rd cells) inhibition of the 7-hydroxy benzimidazole analogs against GSK3β



Analogs	R^1	*n*	Ar	R^3	IC_{50} (μM)	EC_{50} (μM)	$CCIC_{50}$ (μM)	TI[a]
20	4-Cl-phenyl	2	Phenyl	4-$NHSO_2CH_3$	0.007	0.12	28	233
21	4-F-phenyl	2	Phenyl	4-$NHSO_2$-4-tosyl	0.026	0.095	17.5	184
22	4-Cl-phenyl	3	Phenyl	3-OCH_2CO_2H	0.031	0.25	17	68
23	2,4-Di-Cl-phenyl	3	1-Imidazole	—	0.015	0.24	45	187
24	2,4-Di-Cl-phenyl	3	1-Imidazole	4-Cl, 5-Cl	0.006	0.11	60	545
25	3-Cl, 4-F-phenyl	3	1-Imidazole	—	0.003	0.49	>100	>204
26	2-Thiophenyl	3	1-Imidazole	4-Methyl	0.050	0.31	>100	>322
27	2-Thiophenyl	2	Phenyl	4-$CON(C_2H_4)_2NC_2H_5$	0.046	0.40	51	127
28	2-Thiophenyl	3	1-Imidazole	4-Cl, 5-Cl	0.008	0.034	>100	>2941
29	2-Furanyl	3	1-Imidazole	4-Cl, 5-Cl	0.010	0.05	70	1400

[a] In vitro therapeutic index (IC_{50} of cytotoxicity/IC_{50} of enzyme inhibition).

Table 4
Glycogen synthase activation assay

Analogs	EC_{50} (μM) (GS activation, Rd cells)
16	0.013
22	0.080
24	0.170
25	0.100
29	0.040

Table 5
Selectivity assay of the representative analogs

Analogs	Selectivity (remaining activity at 5 μM) (%)							
	GSK3β	IKKβ	ERK1	ERK2	KDR	CDK4	CDK1	AKT
16	−0.58	93.1	73.4	91.4	114	49.2	ND	ND
22	0.3	87.3	80.0	104.5	54.5	58.6	90.9	100.5
24	0.0	80.7	97.9	102.2	108.9	74.9	ND	ND
25	1.0	80.0	66.5	76.0	85.3	52.4	71.3	83.9
27	0.9	72.7	80.9	80.7	60.8	68.2	70.4	96.0
29	−1.7	74.7	53.9	70.6	83.6	38.6	65.2	92.3

ND, not determined.

and R^2-substituents, the aromatic systems for the R^1 and R^2-substituents including the length of linker as well as the substituent effects of both aromatic systems on the inhibitory activities were further investigated. The results were summarized in Table 3. Generally, the propyl linker provided the better enzymatic and cellular inhibitory activities.

The analogs possessing the phenyl substituent for R^1 showed the similar inhibitory potencies regardless of substituent and substitution pattern (**20**, **21**, and **22** for mono-substitution; **23**, **24** and **25** for di-substitution). In addition, the enzyme inhibitory activities were well correlated with the cell based inhibitory activities. Among the analogs possessing the hetero-aromatic moieties for R^1, the analogs possessing the 2-thiophenyl (**28**) and the 2-furanyl (**29**) moieties provided the excellent cellular inhibitory activities. We also examined cellular toxicity of the synthesized analogs.

The MTS assay revealed $CCIC_{50}$s of higher than 100 μM for the analogs **25**, **26** and **28** and the cytotoxic effects at 17 μM for the analogs **21** and **22**. The MTS assay result implied that the 7-hydroxy benzimidazole scaffold is quite safe in terms of the in vitro cytotoxicity.

GSK3β is one of the main mediators of the blood glucose and negatively controls the glycogen synthase. The competitive inhibition of GSK3β against ATP causes an activation of the glycogen synthase and reduces the blood glucose level in mammalian.[17] Accordingly, we tested the glycogen synthase (GS) activation of the representative analogs **16**, **22**, **24**, **25** and **29** to confirm the pathophysiological relation of GSK3β with T2DM.[18] This assay was performed in Rd cell with the 24 h starving serum before the test. The results were summarized in Table 4. Considering that the tested analogs effectively activated the glycogen synthase (GS), the benzimidazole analogs seemed to inhibit the negative modulator (GSK3β) in the GS activation assay system. These results also imply that the benzimidazole analogs lower the blood glucose level of the systemic circulation and accumulate the glycogen in organs.

GSK3β is well known to have multifunction in the many physiological pathways and is ubiquitously expressed in the many types of mammalian tissue, such as liver and muscle tissue.[16] Thus, the kinase selectivity using a broad panel of seven protein kinases was examined for the representative analogs, which depicted excellent activities against GSK3β. The results were summarized in Table 5. In general, the tested benzimidazole analogs exhibited high kinase selectivity and low inhibitory activities against the seven protein kinases at 5 μM level. However, the analogs **16** and **29** partially inhibited CDK4. This is likely due to the structural similarity of the ATP binding pockets of both kinases.[19]

In summary, we established the structure–activity relationship of the 7-hydroxy benzimidazole analogs, which were prepared by the solid phase parallel synthesis. The synthesized benzimidazole analogs exhibited excellent inhibitory activities against GSK3β in both enzyme and cell based assays. In addition, they exhibited the high selectivity in the assay of the seven protein kinases. Currently, further studies for the therapeutic implication of the selected analogs are in progress.

Acknowledgments

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