



Synthesis and structure–activity relationship of 4-quinolone-3-carboxylic acid based inhibitors of glycogen synthase kinase-3 β

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ABSTRACT

The synthesis, GSK-3 β inhibitory activity, and anti-microbial activity of bicyclic and tricyclic derivatives of the 5,7-diamino-6-fluoro-4-quinolone-3-carboxylic acid scaffold were studied. Kinase selectivity profiling indicated that members of this class were potent and highly selective GSK-3 inhibitors.

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Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase having α and β isoforms that are encoded by distinct genes.¹ GSK-3 has been implicated in various diseases including diabetes,² Alzheimer's disease, CNS disorders,³ and cardiomyocyte hypertrophy.⁴ These diseases may be caused by, or may result in, the abnormal operation of certain cell signaling pathways in which GSK-3 plays a role. For example, GSK-3 has been found to phosphorylate and modulate the activity of a number of regulatory proteins, including glycogen synthase, which is the rate-limiting enzyme required for glycogen synthesis. Even though small molecule inhibitors of GSK-3 have been reported in literature⁵ there is, however, a continued need to find more effective therapeutic agents to treat GSK-3 mediated diseases.

As part of an effort to find novel GSK-3 β inhibitors, we conducted a high throughput screen of an internal compound library which led to the identification of compound **1** (Fig. 1) as a 900 nM inhibitor of GSK-3 β .⁶ Interestingly, this compound was originally synthesized as an intermediate during a medicinal chemistry effort to identify 4-quinolone derived anti-microbial agents. As part of a hit-to-lead optimization effort, we set out to improve the potency of this hit by modifying the 7-position as well

as attenuate any anti-microbial activity that might be present in this class of inhibitors.

The synthesis of R⁷ analogs of compound **1** began with nucleophilic displacement of the 5-fluoro group of quinolone **2**⁷ with benzylamine in toluene followed by palladium catalyzed hydrogenolysis to yield amine **4**. Acid hydrolysis of the ester of compound **4** yielded acid **5**. Nucleophilic displacement of the 7-fluoro group of quinolone **5** with a variety of primary amines was accomplished in DMSO at 85 °C to yield the inhibitors shown in Table 1.

Replacement of the R⁷ side chain with selected amines had a significant impact on potency. Incorporation of the branched isopropyl or cyclohexyl amines resulted in derivatives (compounds **6** and **7**, respectively) with similar potency as the original HTS hit. Likewise, use of 3-(2-ethylamino)indole- (**8**) or morpholine (**9**)-containing

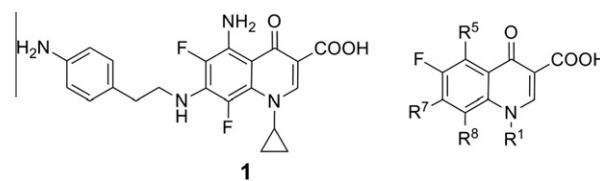


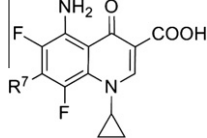


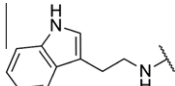
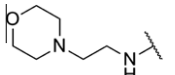
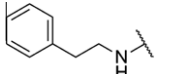
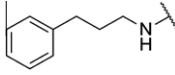
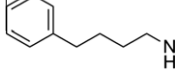
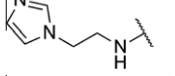
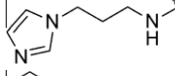
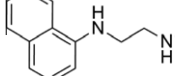
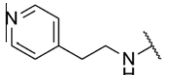
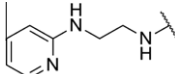
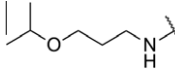
Figure 1. The structure of GSK-3 β HTS hit **1** (GSK-3 β IC₅₀ = 900 nM) and the numbering scheme for the bicyclic 4-quinolone-3-carboxylic acid based inhibitors in this study.

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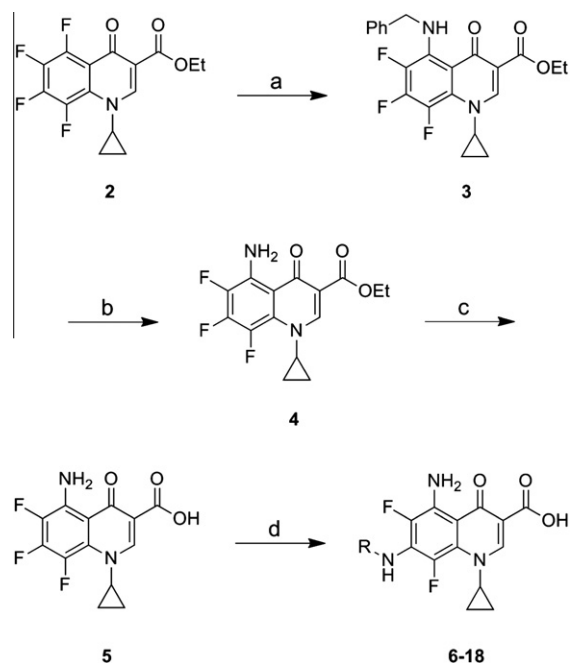
[†] These authors contributed equally to this work.

Table 1
GSK-3 β IC₅₀ values for R⁷ analogs of the HTS hit **1**

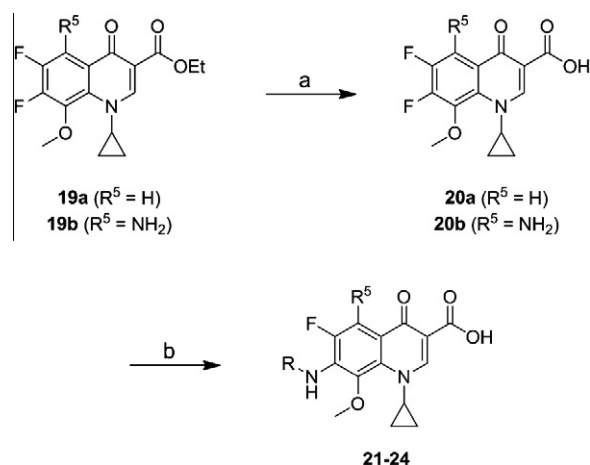
		
Compound	R ⁷	GSK-3 β IC ₅₀ (nM)
6		1200
7		600
8		660
9		2100
10		440
11*		45
12		290
13		3400
14		45
15		47
16		100
17		190
18		110

* Profiled against a panel of human kinases (vide infra).

side chains offered no improvement in potency. To examine the influence of chain length, a series of 7-phenalkyl amino derivatives (**10–12**) were examined and it was found that a chain length of three methylenes (**11**) versus two (**10**) or four (**12**) was found to be optimal. Consistent with this observation was the comparative potency of the 1-imidazole-ethyl-amine derivatives **13** and **14** where compound **14**, which contained linker of three methylenes was a factor of 75 more potent than compound **13**, with a linker length of two methylenes. Other compounds containing selected aryl-containing R⁷ side chains (**15–17**) showed improved potency relative to the HTS hit **1** and exhibited GSK-3 β IC₅₀ values ranging between 40 nM and 200 nM. Interestingly, despite the lack of an aromatic group, derivative **18** was a relatively potent inhibitor with GSK-3 β IC₅₀ value of 110 nM.



Scheme 1. Synthesis of R⁷ analogs of the HTS hit **1**. Reagents and conditions: (a) benzylamine, Et₃N, toluene, 90 °C, 2 h, 82%; (b) H₂, 10% Pd/C, AcOH, 50 °C, 2 h, 89%; (c) AcOH/H₂SO₄/H₂O, reflux, 2 h, 77%; (d) RNH₂, Et₃N, DMSO, 85 °C, 1 h.



Scheme 2. Synthesis of R⁷ amine derivatives of 5-amino and 5-hydro derivatives of 1-cyclopropyl-6-fluoro-8-methoxy-4-quinolone-3-carboxylic acid. Reagents and conditions: (a) AcOH/H₂SO₄/H₂O, reflux (b) RNH₂, Et₃N, DMSO, 85 °C.

Having investigated the 7-position, we turned our exploratory SAR efforts to the primary amine present in the 5-position. Specifically, we synthesized (see Scheme 2) 8-methoxy analogs of **11** and **15** with and without the C5 amine present to assess the impact of this functional group on the inhibitory activity of GSK-3 β (Table 2). The replacement of the 8-F group present in earlier series with a 8-methoxy group was made to minimize the phototoxic potential generally associated with 8-F quinolones.⁸ After the acidic hydrolysis of the 4-quinolone ethyl esters **19a** and **19b**,⁹ nucleophilic displacement of the 7-fluoro group adjacent to the methoxy groups of carboxylic acids **20a** and **20b** with 3-phenyl-propylamine or *N*-(1-naphthyl)ethylenediamine yielded the compounds in Table 2. Comparison of the analogous 5-hydro versus 5-amino derivatives (**21** vs **22**; **23** vs **24**) indicated the presence of the 5-amino group yielded significantly more potent GSK-3 β inhibitors (260- and 840-fold, respectively).

Table 2

GSK-3 β IC₅₀ values for two 8-methoxy-5-amino-4-quinolone-3-carboxylic acid derivatives and their 5-hydro analogs

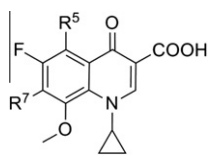
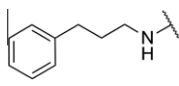
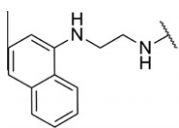
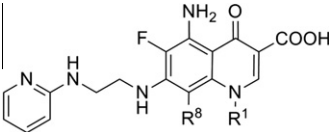




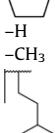
			
Compound	R ⁷	R ⁵	GSK-3 β IC ₅₀ (nM)
21		–H	17000
22		–NH ₂	65
23		–H	26000
24		–NH ₂	31

Table 3

GSK-3 β IC₅₀ values and anti-bacterial activity for bicyclic 4-quinolone-3-carboxylic acid derivatives

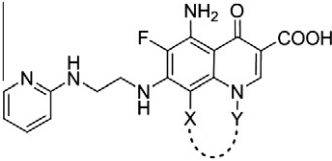
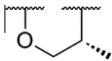
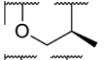
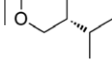
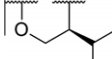
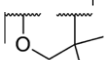
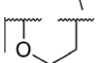

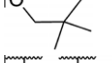
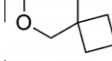
					
Compound	R ⁸	R ¹	GSK-3β IC ₅₀ (nM)	Anti-bacterial activity MIC (μM)	
				<i>E. coli</i>	<i>S. aureus</i>
25	–H		13	0.8	0.2
26	–OCH ₃		160	9	1
27	–OCH ₃		1500	>28	7
28*	–F		22	>29	>29
29	–OCH ₃	–H	1000	25	25
30	–OCH ₃	–CH ₃	270	>13	13
31	–OCH ₃		16000	>28	>28

* Profiled against a panel of human kinases (vide infra).

Because for certain GSK-3 β -related indications, anti-microbial activity may not be desirable, we set out to identify a subset of compounds with potent GSK-3 β inhibitory activity but minimal anti-bacterial activity.¹⁰ A variety of bicyclic quinolones with a fixed R⁷ N-(2-pyridyl)ethylenediamine side chain and varied groups in the 8 and 1-positions were synthesized and evaluated (Table 3). Among these, compounds with a N¹-cyclopropyl group (**25** and **26**) gave the lowest MIC values, a result consistent with the presence of this functionality in quinolone based antibiotics with potent anti-bacterial activity.¹¹ By contrast, two N¹-cyclopentyl derivatives (**27** and **28**) were also investigated and were found to have significantly less anti-bacterial activity. While other 8-methoxy derivatives with N¹-hydro (**29**), N¹-methyl (**30**), and N¹-isopentyl (**31**) groups were shown to have double digit or greater

Table 4

GSK-3 β IC₅₀ values and anti-bacterial activity for bicyclic 4-quinolone-6-carboxylic acid derivatives

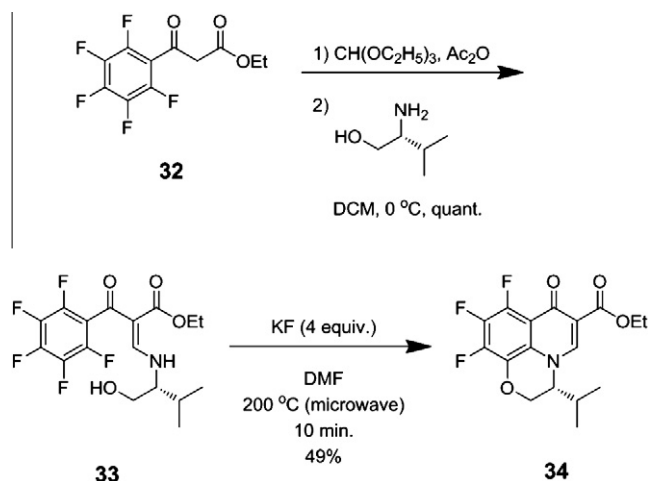
					
Compound	X	Y	GSK-3 β IC ₅₀ (nM)	Anti-bacterial activity MIC (μ M)	
				<i>E. coli</i>	<i>S. aureus</i>
35			560	>243	243
36*			44	4	2
37*			31	>2	>2
38			75	>29	14
39			49	59	59
40			440	>37	>37
41			160	>7	>7
42			57	>36	>36
43*			12	>71	71

* Profiled against a panel of human kinases (vide infra).

μ M MIC values, all of these compounds had GSK-3 β IC₅₀ values significantly higher than 100 nM. It was clear from this study that the interplay between the 8 and 1-positions was critical in identifying the subset of compounds we were targeting.

To further explore this interplay, tricyclic 4-quinolone derivatives (Table 4) were synthesized as shown in Scheme 1, but from a tricyclic starting material, an exemplary synthesis of which is shown in Scheme 3. Treatment of the ketoester **32** with triethylorthoformate followed by (*R*)-valinol yielded the intermediate imine **33**. The cyclization of this material was accomplished using potassium fluoride as a base and microwave irradiation¹² to yield the trifluoroquinolone **34**. This compound was further elaborated as shown in Scheme 1 to yield the inhibitor **37** (Table 4).

Selected examples of these tricyclic quinolones yielded sub-100 nM GSK-3 β inhibitors and minimal anti-bacterial activity. The 3-(*R*)-methyl derivative **35** was found to be a relatively poor GSK-3 β inhibitor (GSK-3 β IC₅₀ = 560 nM) but have essentially no anti-bacterial activity. By contrast, its enantiomer **36** was 13 times more potent against GSK-3 β (GSK-3 β IC₅₀ = 44 nM) with single digit micromolar MIC values. In the case of the analogous chiral isopropyl derivatives **37** and **38**, the difference in potency was not as pronounced with GSK-3 β IC₅₀ values of 31 nM and 75 nM, respectively. Substituted achiral derivatives were also investigated including the 3,3'-gem-dimethyl derivative **39** which was found to have a GSK-3 β IC₅₀ value of 49 nM. Moving this gem-dimethyl group to the 2-position (**40**) or expanding the saturated ring of compound **39** (i.e., compound **41**) resulted in derivatives that were significantly less potent



Scheme 3. Synthesis of the tricyclic trifluoroquinolone **36**, the starting material used to produce compound **37** according to Scheme 1.

Table 5

Kinase selectivity profiling (KiNativ™) of selected GSK-3β inhibitors in HL60 lysate showing % inhibition of the indicated kinase(s) at a screening concentration of 10 μM

Kinase	Family	11	28	43	37	36
GSK-3α	CMGC	93.6	> 99	> 99	96.7	> 99
GSK-3β	CMGC	82.2	97.3	97.2	95.9	98.2
ABL1,2	TK	48.3	< 35	< 35	< 35	< 35
AMPKα1,2	CAMK	< 35	43.9	< 35	< 35	< 35
CDK10	CMGC	< 35	< 35	< 35	64.9	< 35
CDK2	CMGC	< 35	< 35	< 35	< 35	< 35
CDK5	CMGC	< 35	< 35	< 35	< 35	< 35
CDK6	CMGC	< 35	< 35	< 35	< 35	< 35
CDK7	CMGC	< 35	< 35	< 35	< 35	< 35
CDK9	CMGC	< 35	< 35	< 35	< 35	< 35
CHED	CMGC	< 35	< 35	< 35	< 35	< 35
DYRK1B	CMGC	< 35	< 35	< 35	< 35	< 35
Erk1/2	CMGC	< 35	< 35	< 35	< 35	< 35
JNK1,2,3	CMGC	< 35	< 35	< 35	< 35	< 35
LKB1	CAMK	< 35	43	53.6	48.1	63.4
p38α	CMGC	< 35	< 35	< 35	< 35	< 35
p38δ	CMGC	< 35	< 35	< 35	< 35	< 35
p38δ/γ	CMGC	< 35	< 35	< 35	< 35	< 35
PITSLRE	CMGC	< 35	< 35	< 35	40.2	< 35
PRKDC	Atypical	< 35	< 35	48.1	< 35	< 35
ULK3	Other	63.3	< 35	< 35	< 35	< 35

GSK-3β inhibitors. Replacement of the gem-dimethyl group of compound **39** with cyclobutyl (**42**, GSK-3β IC₅₀ = 57 nM) was well tolerated. When replaced with cyclopentyl (**43**) the resulting derivative was found to be not only the most potent GSK-3β inhibitor in this study (GSK-3β IC₅₀ = 12 nM), but also have minimal anti-microbial activity (MIC ≥ 71 μM).

Selected potent GSK-3β inhibitors (compounds **11**, **28**, **36**, **37**, and **43**) were profiled against a panel of kinases using a desthiobiotin, acyl-phosphate ATP probe as previously described.¹³ Post probe-labeling of HL60 lysate in presence of inhibitors (10 μM) fol-

lowed by trypsinization, kinase active site peptides were identified and quantified using LC-MS/MS. Percent inhibition was calculated by the decrease in the signal from each peptide relative to the intensity seen in the absence of inhibitor. Over 90 kinases were profiled including both GSK-3 isoforms, and selected data is shown in the form of a heat map in Table 5.¹⁴ Notably, all 5 GSK-3β inhibitors were found to be highly selective for GSK-3, even when screened against kinases from the CMGC family, in which both GSK-3 isoforms are members. The only off-targets observed had relatively weak inhibitory activity compared to GSK-3, with the predicted affinity (based on the percent inhibitions observed at the screening concentration) expected to be in the μM range.

In conclusion, the hit-to-lead modification of the HTS hit **1** yielded a series of sub-μM, tricyclic GSK-3β inhibitors based on the 5,7-diamino-6-fluoro-4-quinolone-3-carboxylic acid scaffold. Several potent GSK-3β inhibitors were determined to be highly selective for GSK-3 when profiled against over 90 kinases. Additional SAR and modifications to improve the potency of this series will be presented in due course.

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- Other kinases that gave <35% inhibition but are not shown in Table 5: AKT1, AKT2, DMPK1, MSK1, p70S6K, PKAα/PKAcγ, PKCα/PKCβ, PKN2, ROCK1/2, RPS6K1, SGK3, EEF2K, CaMK2γ, CHK1, DCAMK1, MARK3, PHKγ2, PKD1/2, PKD2, RSK1/RSK2/RSK3, RSK4, CK1α, FRAP, PIK4CA, PIP5K2α, SMG1, AurA, AurA/AurB/AurC, IKKα, IKKβ, IRE1, MPK1, NEK6/NEK7, NEK9, NEK9, PEK, PKR, PLK1, Wee1, Wnk1/Wnk2/Wnk4, HSER, HPK1, KHS1/KHS2, LOK, MAP2K1, MAP2K4, MAP3K2, MAP3K5, MST1, MST1, MST2, MST4/YSK1, OSR1, SLK, STK15, TAO1/3, TAO2, ABL1/ABL2, CSK, FER, FES, FGR, JAK1, LYN, PYK2, SYK, ARAF, BRAF, IRAK4, RIPK3, TAK1, ZAK.