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Syntheses, Neural Protective Activities, and Inhibition of Glycogen Synthase Kinase-3 β of Substituted Quinolines

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Abstract

A new series of fifteen 5-, 6-, and 8- appended 4-methylquinolines were synthesized and evaluated for their neural protective activities. Selected compounds were further examined for their inhibition of glycogen synthase kinase-3 β (GSK-3 β) and protein kinase C (PKC). Two most potent analogs, compounds **3** and **10**, show nanomolar protective activities in amyloid β -induced MC65 cells and enzymatic inhibitory activities against GSK-3 β , but poor PKC inhibitory activities. Using normal mouse model, the distribution of the most potent analog **3** in various tissues and possible toxic effects in the locomotors and inhibition of liver transaminases activities were carried out. No apparent decline of locomotor activity and no inhibition of liver transaminases were found. The compound appears to be safe for long-term use in Alzheimer's disease mouse model.

Keywords

Alzheimer's disease; amyloid β ; glycogen synthase kinase-3 β ; neural protective activity; protein kinase C; quinolines

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the most common form of dementia in the elderly.¹ AD brains have three major lesions, (1) formation of

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Supplementary data: Supplementary data (synthetic procedure, chemical analysis data, enzyme assays, cell assays, *in vivo* toxicity study, and quantification of tested compound in various tissues) associated with this article can be found, in the online version, at <http://dx.doi.org>

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aggregates and plaques of amyloid- β ($A\beta$) peptide, a 39-43 residue protein, outside of neurons; (2) formation of abnormal neurofibrillary tangles (NTFs) consists of hyperphosphorylated tau protein inside the neurons; and (3) dysfunction of neurons including cholinergic, noradrenergic, serotonergic and pyramidal neurons.² Major strategies for experimental disease-modifying therapies include targeting multiple sites in $A\beta$ metabolism ($A\beta$ synthesis, aggregation, deposition and clearance), targeting tauopathy (modulation of tau phosphorylation and inhibition of tau aggregation), regulating cholesterol homeostasis, anti-excitotoxicity, anti-inflammation and modulating calcium homeostasis, among others.¹⁻³ A number of compounds has shown efficacy in AD transgenic mouse models,⁴⁻¹² but their efficacy in AD patients remains uncertain. Moreover, several drug candidates in clinical trials failed to show benefits.¹⁻³ In our pursuit of finding neural protective compounds for AD, we use MC65 cells protection assay as our primary screen for bioactive compounds,¹³⁻¹⁵ and a class of quinolines containing various substituents (Figure 1) were found to have submicromolar activities. MC65 cells assay generates few false positive results and identifies leads that penetrate cells and ameliorate $A\beta$ oligomer-induced toxicity.¹³ Moreover, studies of the enzyme assay inhibition of glycogen synthase kinase-3 β (GSK-3 β) revealed several quinoline molecules inhibited the enzyme in nanomolar ranges. GSK-3 β is abundant in neurons of the central nervous system and catalyzes the phosphorylation of tau proteins, which are overexpressed in AD brains.¹⁶ Hyperphosphorylation of tau proteins in brain from imbalanced activity of GSK-3 β results in neurofibrillary tangles and leads to cell death.¹⁶⁻¹⁸ Moreover, the “dual pathway” hypothesis¹⁹ suggests that GSK-3 affects both hyperphosphorylation of tau and elevation of $A\beta$ via the enhancement of enzymatic processing of APP. A dual effect of reduction of $A\beta$ toxicity and inhibition of GSK-3 β may provide a greater beneficial effect than conventional GSK-3 β inhibitors and anti- $A\beta$ molecules.²⁰ Herein, we reported the syntheses of quinolines **2** - **16** (Figure 1) and evaluation of their bioactivities along with our previously reported quinoline **1**.²¹ Several compounds possess dual effects of inhibition of $A\beta$ toxicity in MC65 cells and GSK-3 β enzyme.

From an initial screening of synthesized substituted quinoline compounds using MC65 cells, we found that compound **1** possesses strong neuronal cell protective activity (*vide infra*). Hence, its analogs, quinolines **2** – **16** possessing different heterocycle- or arene-appended methylamino moiety at C8, different C5 aryloxy moieties, no C5 aryloxy group, and C6 hydroxyl function, were synthesized. A reductive amination reaction was used to assemble compounds **2** – **7** via the coupling of amine **17**²¹ and aldehydes **18** – **23** with sodium cyanoborohydride in methanol in good to moderate yields (Scheme 1). Aldehydes **18** – **22** were obtained from commercial sources while **23** was prepared as reported.²² Compounds **2** – **7** contain different heterocycle- or arene-appended methylamino functions at C8, which depart from that of compound **1**.

The C8 appended 3-aminopropylamine function of compound **1** was also modified by introducing a 3-amino-3-oxopropylamine moiety at C8, i.e., compound **8**, or varied by a 2-furanylamide group, compound **9**. Compound **8** was readily synthesized from a Michael addition reaction of amine **17** with acrylamide in acetonitrile in a sealed tube at 120°C in a 57% yield, and amide **9** was prepared in a 75% yield from the acylation reaction of amine **17**

with 2-furancarboxyl chloride (**24**) (Scheme 2). Among the tested quinolines using MC65 cell assays, compounds **1** and **3** appear to be the most active quinolines (*vide infra*), hence the C6-hydroxyl analog of **3** and molecules possessing different substituents at C5, i.e., compounds **10** - **16**, were examined. The C6-methyl ether group of **17** was removed with boron tribromide in dichloromethane, and the resulting quinoline **25** condensed with aldehyde **19** to give hydroxyl analog **10** in a 52% yield.

C5-Aryloxy analogs of **1** such as compounds **11** and **13** were obtained from a sequence of reactions starting from bromide **26** (Scheme 3).²¹ Hence, nucleophilic aromatic substitution reactions of bromide **26** with potassium 3-fluorophenoxide (**27**) and potassium phenoxide (**28**) separately in DMF gave aryl ether **29** and **30** in 65 and 80% yield, respectively (Scheme 3). Removal of the acetyl protecting group of **29** and **30** with HCl in ethanol followed by ring closing reactions with 3-buten-2-one and arsenic acid in phosphoric acid provided quinolines **31** and **32** in 35 and 45% yield, respectively. Reduction of the nitro function of **31** and **32** separately with iron in acetic acid followed by alkylation of the resulting 8-aminoquinolines **33** and **34** with 3-iodopropylphthalimide,²¹ and deprotection with hydrazine afforded **11** and **13**, respectively. Condensation of amines **33** and **34** separately with 4-hydroxybenzaldehyde and sodium cyanoborohydride furnished amines **12** and **14**, respectively.

Quinolines without C5 aryloxy function, **15**²³ and **16**, were similarly synthesized from the ring closing reaction of 4-amino-3-nitroanisole with 3-buten-2-one followed by the reduction with iron in acetic acid to give amine **35**²⁴ in an 85% overall yield (Scheme 4). Alkylation of amine **35** with 3-iodopropylphthalimide followed by the treatment of hydrazine gave C5-H analog **15**, and reductive amination of amine **35** with aldehyde **19** and sodium cyanoborohydride afforded derivative **16**.

MC65 cell line was used as a screen to search for neural protective compounds against cell death induced by oligomeric A β peptides.¹³⁻¹⁵ On day three, the induction of S β C gene (in the absence of tetracycline) in MC65 cells leads to the production of the C99 fragment of amyloid precursor protein and subsequently to A β peptides from the proteolysis by γ -secretase, resulting in cell death. Compounds that protect cell from death likely possess anti-A β property. An addition of tetracycline, an antioxidant, to the media of MC65 cells suppresses the production of C99 fragment and cells survive. Consequently, toxicity of the compound to MC65 cells is determined. Table 1 tabulates the EC₅₀ (median effective concentration) and TC₅₀ (median toxic concentration) values of quinoline compounds, **1** - **16**. From our initial screen of compounds **1** - **9** having various functionalities at C8 of the quinoline, compounds **1** and **3** showed the greatest activities with EC₅₀ values of 0.15 and 0.12 μ M, respectively. Results suggest that the presence of a 3-aminopropylamine or *p*-hydroxyphenylmethylamine function leads to higher bioactivity. Modifications at C5 and C6 were therefore carried out by keeping appended C8 with either 3-aminopropylamine or *p*-hydroxyphenylmethylamine, such as compounds **10** - **16**. Among these seven derivatives, C6-hydroxy derivative **10** and C5-phenoxy **13** are the most active analogs with EC₅₀ values of 0.30 and 0.42 μ M, respectively, while other analogs having EC₅₀ values range from 0.53

to 17.6 μM . The results indicate that compound **3** is a suitable candidate for mechanistic investigation.

Hyperphosphorylation of tau proteins by GSK-3 β leads to the formation of neurofibrillary tangles,¹⁶⁻²⁰ hence inhibition of GSK-3 β by selected quinoline compounds was conducted in search of possible mechanism of action. Protein kinase C (PKC) mediates the function of other proteins in signal-transduction pathways of different cell types through the phosphorylation of hydroxyl functions of serine and threonine residues of these proteins.²⁶ In particular, phosphorylation of potent activators of transcription would increase oncogene expressions resulting in the promotion of cancer progression.²⁷ Therefore, the inhibition of PKC was also carried out. Table 2 summarizes results of the inhibition of GSK3 β and PKC and the selectivity index (SI) by selected quinolines using the respective GSK-3 β kinase assay and PepTag® non-radioactive PKC assay kits (both obtained from Promega Co.). Interestingly, the gap junction enhancer compound **1**²¹ inhibited PKC with half maximum inhibitory concentration (IC₅₀) value of 35 nM but does not inhibit GSK-3 β up to 1 mM. A similar finding is observed with quinoline **7** except with much higher IC₅₀ value of 400 μM against PKC. Quinolines **3** and **10** on the other hand show excellent selectivity for GSK-3 β . The IC₅₀ values of **3** and **10** against GSK-3 β are 35 nM and 158 nM, respectively, while the values against PKC are 240 and 750 μM , respectively. The SI values of these two compounds are 6,857 and 4,747, respectively, suggesting kinase specificity. Under similar assay conditions, staurosporine^{26,28} show potent but non-discriminating inhibitions with IC₅₀ values against GSK-3 β and PKC of 38 and 33 nM.

Compound **3** possesses strong neuronal cell protection and inhibitory activity against GSK-3 β , therefore, to examine whether **3** is suitable for future *in vivo* efficacy study, the distribution of **3** in various tissues and possible toxic effects in the locomotors and inhibition of liver enzymes activities in mice were carried out. The amounts of **3** in various tissues were quantified by following a reported procedure,²⁹ and results are summarized in Table 3 from an oral gavage administration each day of 5 mg/Kg body weight of CD1 mice of **3** (two untreated and four treated mice were used) for 75 days. The amounts of **3** in various organs and plasma remained in low concentrations after 75 days and appeared to accumulate in brain and pancreases in greater amounts than other tissues and plasma.

After 75 days of treatment, behavioral activity was monitored using Versamax (AccuScan Instruments Inc., Columbus, OH, USA), where readings were taken before and after administration of **3**. The Versamax chamber is ventilated and equipped with infrared sensors along the side wall. Prior to the reading, each mouse was left in the chamber for 15 minutes for acclimatization. Administration of **3** for 75 days did not showed any effect on the overall locomotors activity of mice in terms of clockwise or counter-clockwise activity, total covered distance, rest time, vertical activity, and horizontal activity when compared with control mice (Figure 2). Results suggest that chronic administration of **3** did not have any behavioral side effects in mice.

Possible liver toxicity was examined by measuring enzymatic activities of liver transaminases, aspartate aminotransferase (AST) and alanine transaminase (ALT), biomarkers of toxicity. Mice were sacrificed on day 75 after daily oral administration of **3**.

Blood was collected from heart by using syringe containing heparin to prevent clotting. Plasma was separated immediately to prevent hemolysis by centrifugation at $10,000 \times g$ for 5 minutes. The enzymatic activities of AST and ALT were determined using a commercially available kit (Pointe Scientific, Inc, Canton, MI, USA), according to manufacturer's instructions. To evaluate the toxicity of **3** upon chronic administration, ALT and AST enzyme activities were evaluated in the plasma of control and treated mice, and results are depicted in Figure 3. No significant difference in the activities of ALT and AST was observed between control and treated mice suggesting that **3** is relatively safe for long-term use.

In summary, a number of neural protective quinoline molecules were synthesized and evaluated in cells and enzyme assays. Compound **3** and its C6-hydroxyl analog **10** were the most potent molecules and they inhibit GSK-3 β selectively in nanomolar concentrations. Compound **3** distributed mainly to the brain and pancreases in mice after administration of 5 mg/Kg daily by oral route over 75 days and showed no apparent toxicity through the examination of locomotors activity and liver transaminases. Hence, the molecule appears to be safe for long-term study in AD mouse model. The synthetic procedure leading to this class of neural protective compound is general and further structural optimization is possible for future drug discovery and development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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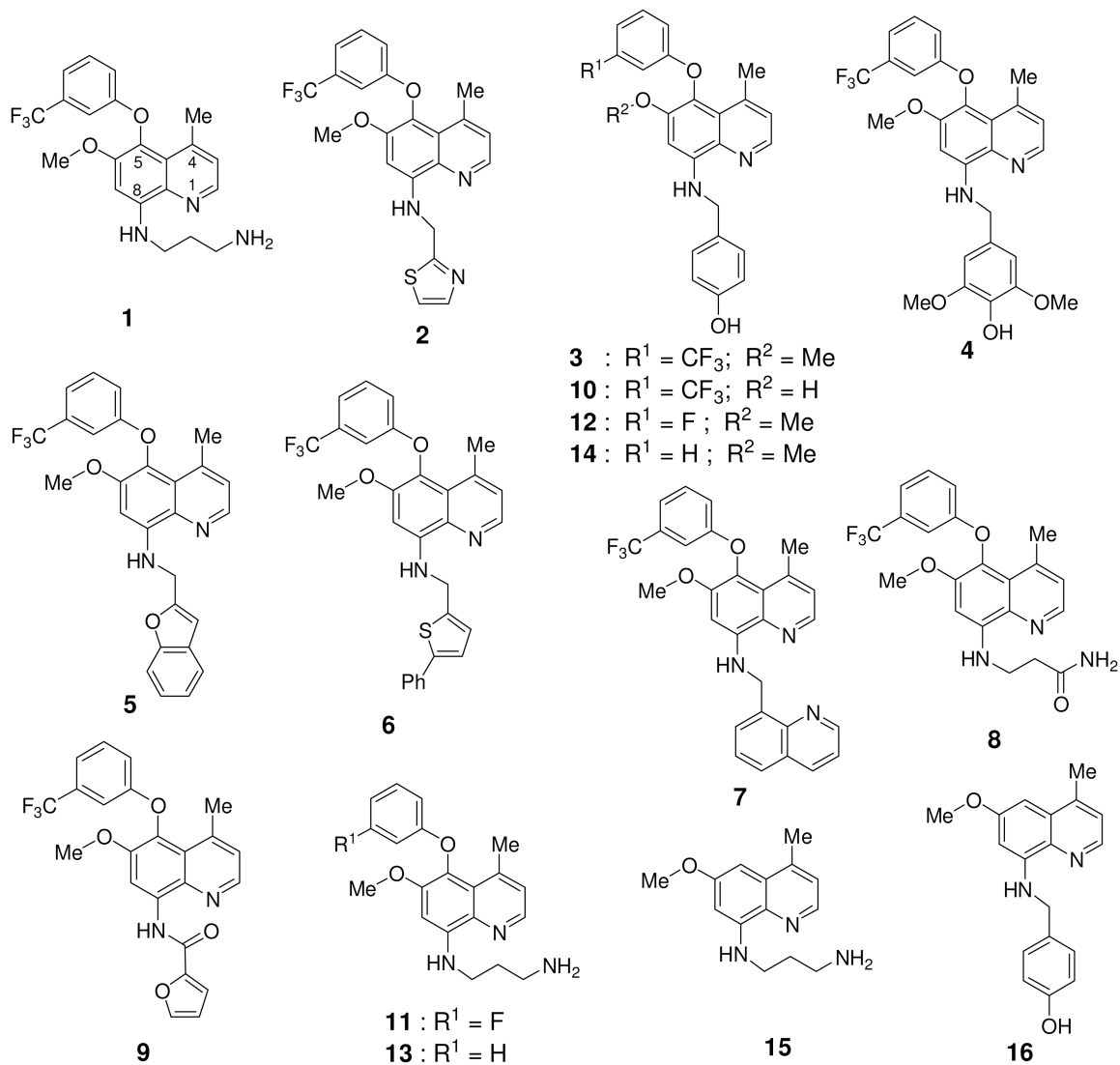


Figure 1.
Synthesized and bioevaluated quinolines **1** - **16**.

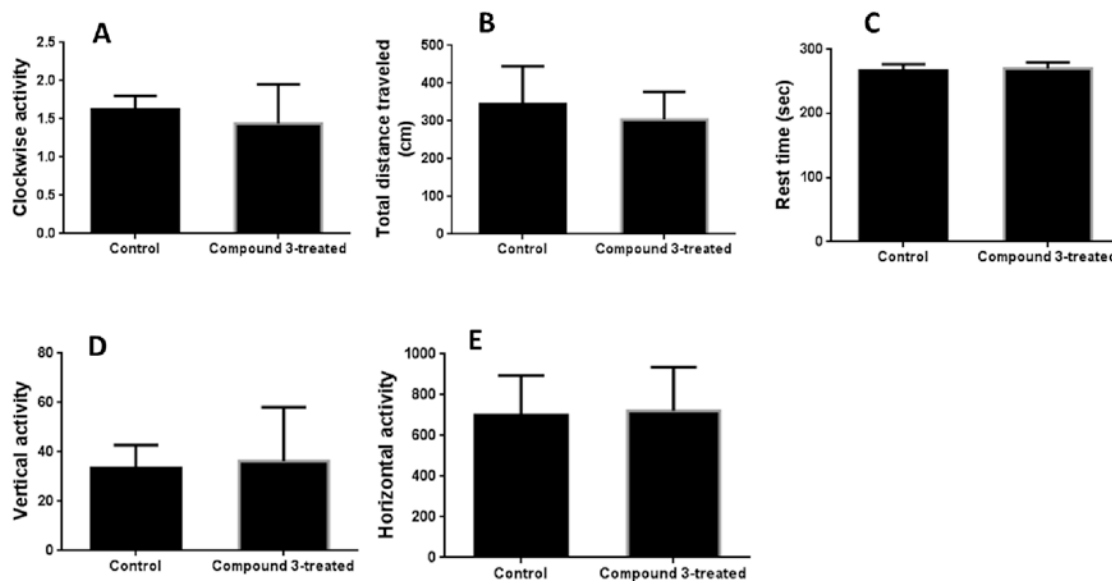


Figure 2. Effect of chronic administration of compound **3** on the locomotor activity of mice. Control and compound **3** (5mg/Kg daily by oral gavage for 75 days) treated mice were acclimatized for 15 min inside Versamax chamber followed by monitoring their movements. (A) Clockwise revolution, (B) total distance covered, (C) rest time, (D) vertical activity, and (E) horizontal activity.

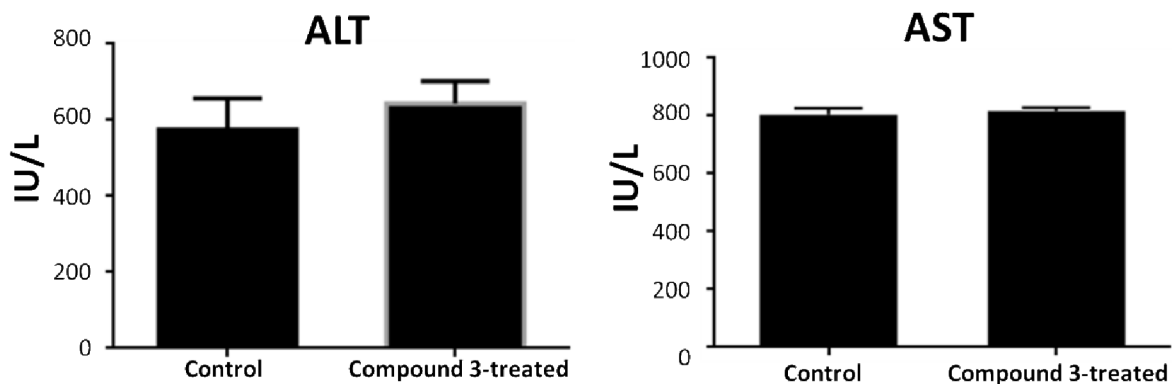
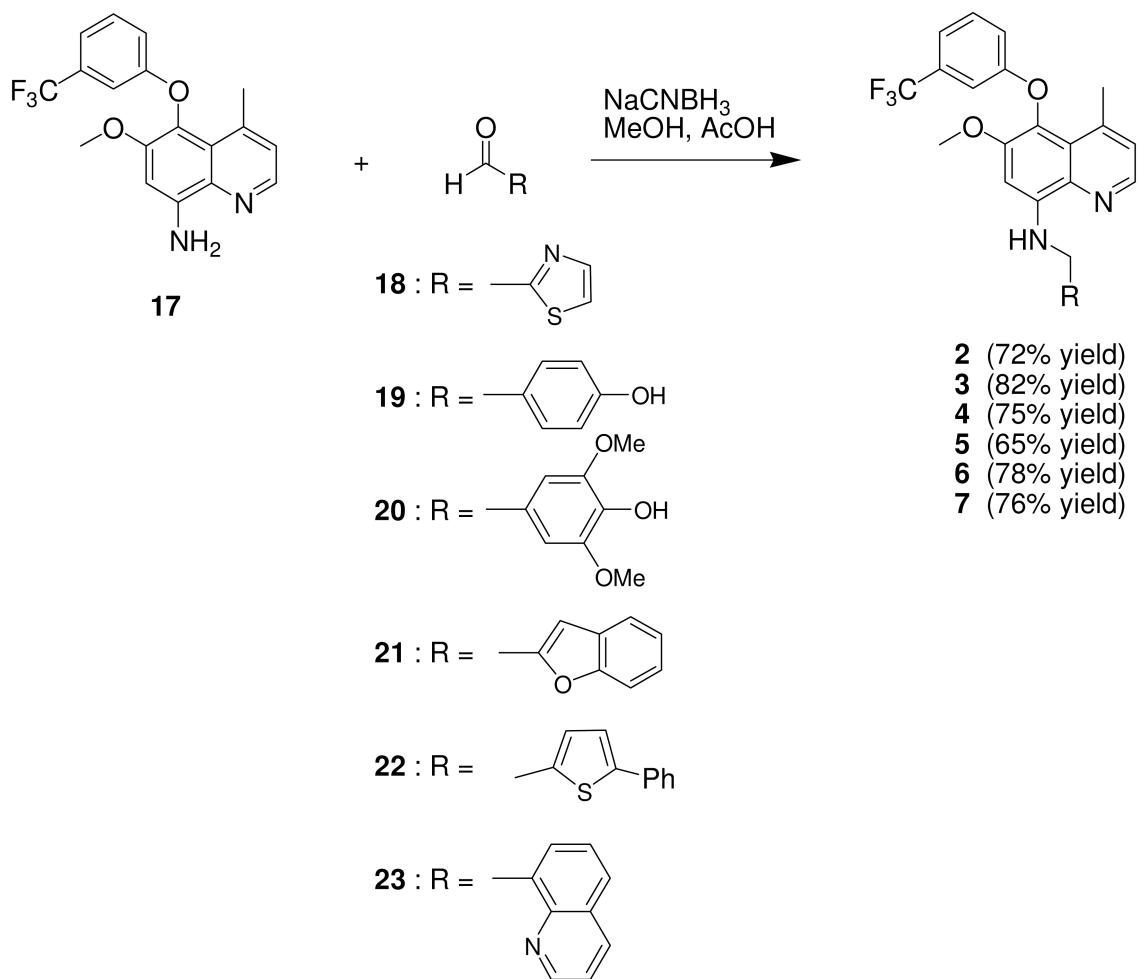
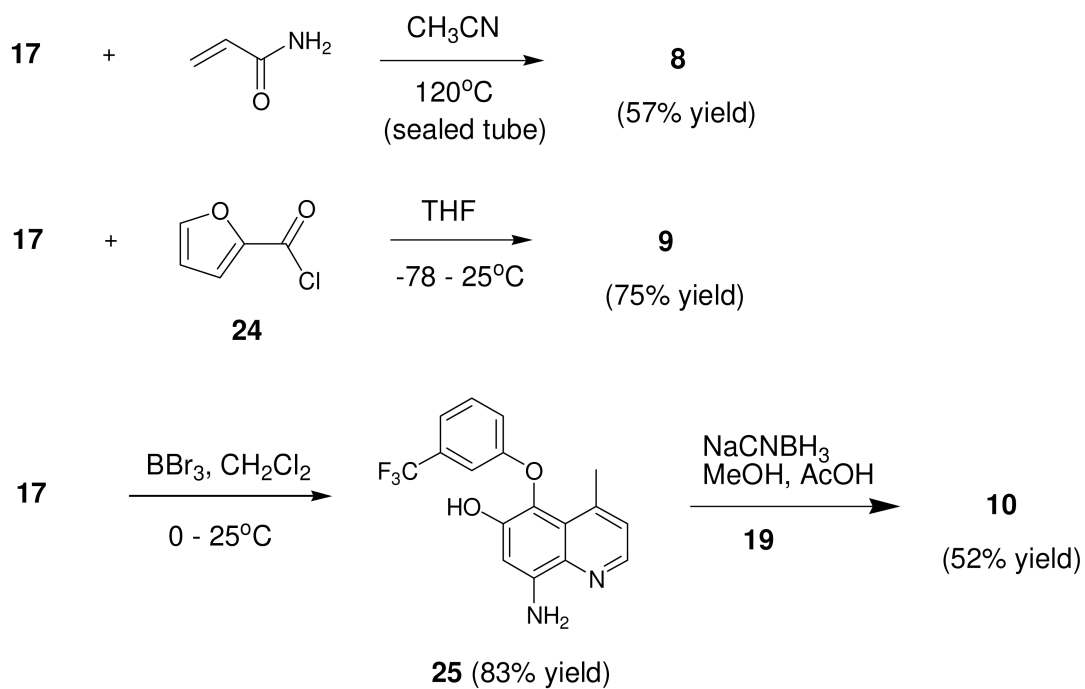


Figure 3.

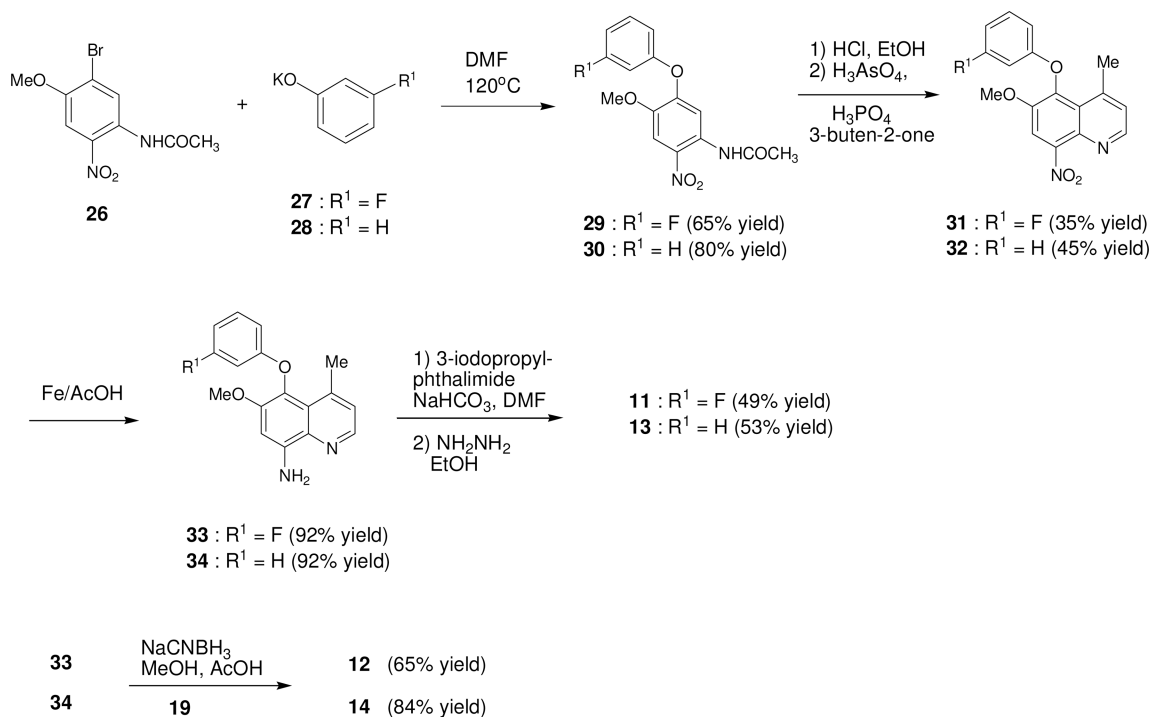
Effect of compound **3** on the biochemical markers of toxicity. After 75 days of daily administration of **3** (5 mg/kg) by oral gavage, mice were sacrificed and plasma was separated from the blood of control and treated mice and evaluated for ALT and AST enzymatic activities.



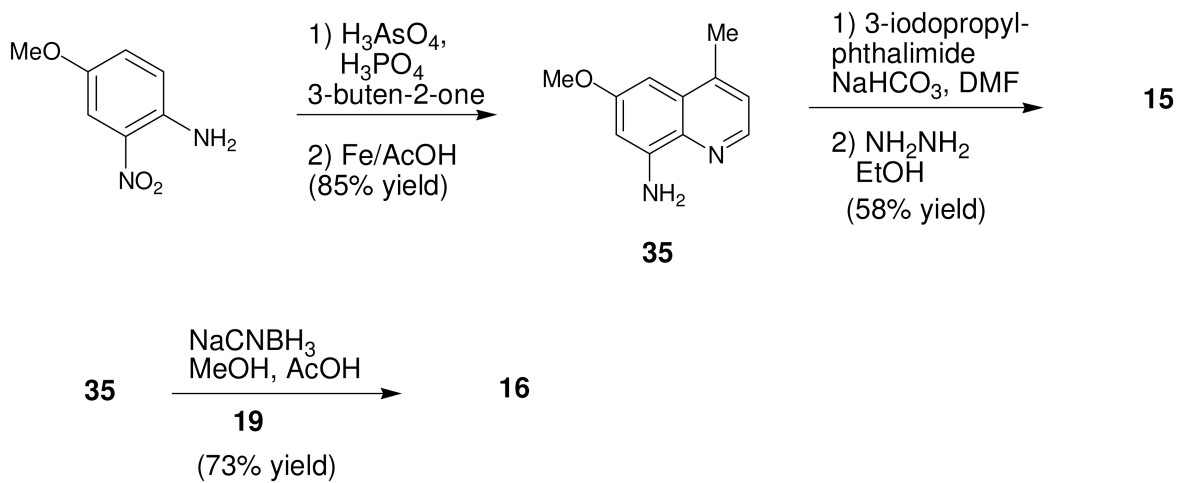
Scheme 1.
 Synthesis of Compounds **2 - 7** via reductive amination reactions.



Scheme 2.
Synthesis of Quinolines **8** - **10**.



Scheme 3.
 Synthesis of Quinolines **11 - 14**.



Scheme 4.
Synthesis of Quinolines **15** and **16**.

Table 1

EC₅₀ and TC₅₀ values of Compounds **1** – **16** from MC65 cell protection assays.²⁵ Values are expressed in mean ± standard deviation.

Compound	EC ₅₀ (μM)	TC ₅₀ (μM)	Compound	EC ₅₀ (μM)	TC ₅₀ (μM)
1	0.15 ± 0.02	2.10 ± 0.03	9	3.47 ± 0.32	>50
2	2.39 ± 0.06	20.32 ± 1.22	10	0.30 ± 0.01	3.31 ± 0.30
3	0.12 ± 0.01	1.38 ± 0.08	11	0.60 ± 0.02	7.28 ± 0.26
4	0.48 ± 0.03	2.91 ± 0.15	12	0.70 ± 0.08	2.60 ± 0.20
5	0.46 ± 0.11	>50	13	0.42 ± 0.01	8.16 ± 0.01
6	0.39 ± 0.02	14.50 ± 1.68	14	0.53 ± 0.01	4.01 ± 0.38
7	0.19 ± 0.02	>50	15	17.62 ± 0.37	>50
8	0.50 ± 0.02	>50	16	2.62 ± 0.15	20.54 ± 0.21

Table 2

Enzyme inhibitory activities of GSK-3 β and PKC by selected quinoline compounds and selectivity index (SI) values.²⁵ Values are expressed in mean \pm standard deviation.

Compounds	IC ₅₀ value, GSK-3 β	IC ₅₀ value, PKC	SI (PKC IC ₅₀ /GSK3 β IC ₅₀)
1	> 1 mM	35 \pm 8 nM	< 3.5 \times 10 ⁻⁵
3	35 \pm 6.36 nM	240 \pm 21.2 μ M	6,857
7	> 1 mM	400 \pm 13.7 μ M	< 0.4
10	158 \pm 19.1 nM	750 \pm 9.3 μ M	4,747
Staurosporine	38 \pm 7 nM	33 \pm 5 nM	0.87

Table 3

Distribution of compound **3** in various tissues and plasma from an oral route administration of **3** into CD1 mice with 5 mg/Kg body weight daily for 75 days (n = 4). Values are expressed in mean \pm standard deviation, and ND is not detectable.

Tissues	Brain	Pancreases	Liver	Kidney	Lung	Plasma
Concentration of 3 (μ M)	24.4 \pm 6.4	26.5 \pm 6.6	7.1 \pm 0.6	16.1 \pm 2.1	ND	4.5 \pm 0.3