Synthesis and Evaluation of [2-(4-Quinolyloxy)phenyl]methanone Derivatives: Novel Selective Inhibitors of Transforming Growth Factor-β Kinase

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We synthesized and evaluated various [2-(4-quinolyloxy)phenyl]methanone derivatives. These compounds had novel chemical structures that were distinct from those of previously reported inhibitors. Biological data suggested that these compounds inhibited transforming growth factor-β signaling by interacting with the ATP-binding pocket of the transforming growth factor-β type I receptor kinase domain. Here, we report on the synthesis and structure−activity relationships of the compounds in this series.

Introduction

Transforming growth factor-β (TGF-β) is a cytokine that plays important roles in cell differentiation, wound healing, and regeneration. TGF-β signaling is mediated by two types of transmembrane receptor serine/threonine kinase: types I (RI) and II (RII).[1,2] TGF-β binding to RII recruits and activates the TGF-β RI kinase (activin receptor-like kinase 5 (ALK5)). Activated ALK5 phosphorylates a subset of downstream signaling molecules, Smad2 and Smad3, allowing them to bind to the commonly mediated Smad4.[1,2] This complex is shuttled into the nucleus and regulates the transcription of target genes.[1,2] The deregulation of TGF-β signaling has been implicated in the pathogenesis of various diseases, including fibrosis,[3] atherosclerosis,[4] and cancer.[5–7] The inhibition of TGF-β signaling with a small-molecule compound is thus expected to be a practical method for the treatment of the diseases mentioned above. Some research groups have reported on small-molecule inhibitors of the TGF-β receptor kinase.[8] The chemical structures of many of these inhibitors possess a similar topology, comprising a five-membered azole scaffold attached by two or three aryl groups.

We have previously reported on several inhibitors of receptor tyrosine kinases, including the platelet-derived growth factor receptor (PDGFR),[9] fibroblast growth factor receptor 2 (FGF-R2),[10] and vascular endothelial growth factor receptor 2 (VEGFR-2)[11] (Figure 1). Our initial screening for TGF-β signaling inhibitors using the Smad2/3 reporter assay identified some of our in-house quinoline compounds as potential hits. Among these, **6** was selected as a seed compound after an in vitro nonspecific toxicological study (Figure 1).

The resulting series of compounds inhibited the TGF-β RI kinase in an ATP-competitive manner,[12] similar to all of the quinoline derivatives previously reported by our group. Our preliminary computational modeling using **6** and the kinase domain of TGF-β RI suggested the presence of an essential hydrogen bond between the nitrogen atom at the 1-position of quinoline and the backbone N−H group in the hinge region of the kinase domain. This interaction appeared to mimic the binding of an ATP molecule to the pocket. Interestingly, the reduction of the carbonyl group to an alcohol group or to an alkyl group significantly reduced the potency. This observation suggested a requirement for a hydrogen bond acceptor at the 2-position to the quinolyloxy group on the phenyl ring. On the basis of our experience and knowledge of kinase inhibitors and **6**, we initiated a research program targeting selective inhibitors of the TGF-β RI kinase.

Chemistry

The representative processes used to synthesize [2-(4-quinolyloxy)phenyl]methanone derivatives are illustrated in Schemes 1 and 2. 4-Chloro-6,7-dimethoxyquinoline (**7**) was prepared as previously reported,[11] and a coupling reaction with the phenols, purchased or synthesized, produced the phenoxyquinoline derivatives (Scheme 1). 2-(4-Quinolyloxy)benzaldehyde (**9a**) was prepared using 2-hydroxybenzaldehyde under the same coupling reaction conditions. The Grignard reaction followed by oxidation produced compounds with a range of alkylphenylketones, such as **9b** (Scheme 2).

Results and Discussion

All new compounds were tested in the Smad2/3 reporter assay. Initial structure−activity relationship (SAR) studies were started with the introduction of small groups to the benzophenone moiety. As shown in Table 1, the introduction of a methyl group at R1 increased the potency of **9c**. A similar tendency was observed with halogen analogues **9d** and **9e**, although the improvement in activity was not significant. Preliminary computational modeling suggested that these small groups occupied a hydrophobic pocket unique to TGF-β receptors. Interestingly, the methoxy group of **9f** led to a significant reduction in activity. The introduction of small groups at R2 caused a slight decrease in activity (**9g**, **9h**, and **9i**), while significantly reduced activity was observed when a bulky group was attached at R3 in **9j**.

In order to improve the druglike features of these compounds, acetophenone rather than benzophenone derivatives were synthesized and tested in the Smad2/3 reporter assay. Table 2 shows the SARs of compounds with various acyl groups at the 2-position of the phenyl ring. Even small alkyl groups, such as ethyl (**9m**), could replace the phenyl ring of **9c** without a loss of activity, while the benzaldehyde analogue **9k** showed comparatively little inhibition. Sterically hindered alkyl groups (**9n**) and heterocyclic groups (**9p** and **9q**) caused a slight decrease in the activity. None of the amide derivatives, as represented by **9o**, showed significant inhibition.

Next we examined the substituent effect at the 4-position and 5-position on the phenyl ring. The results are summarized in

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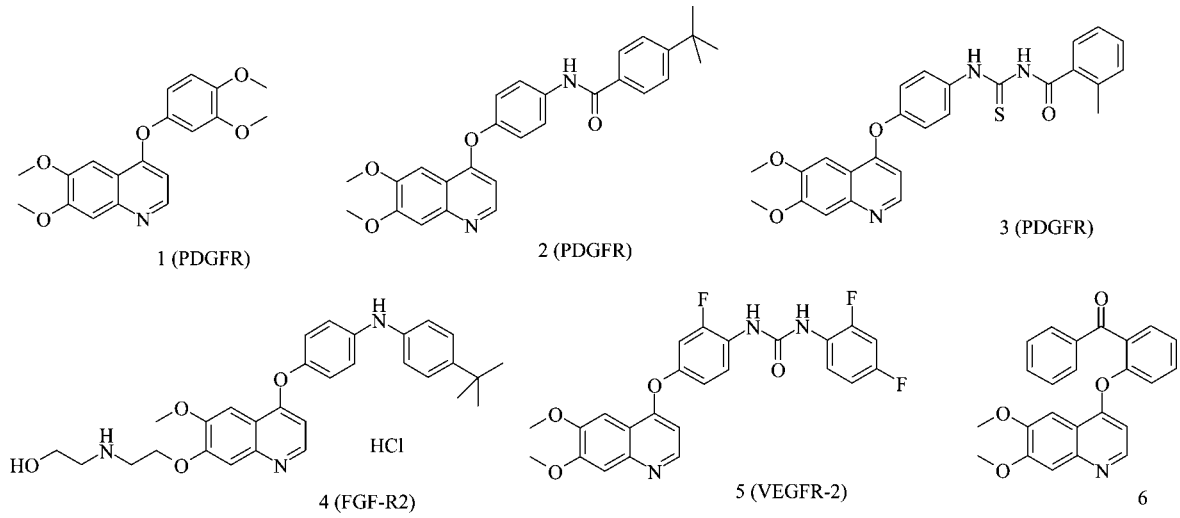
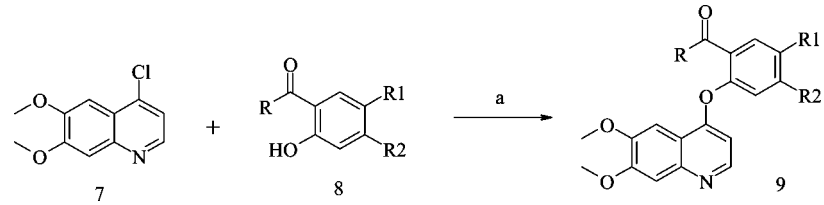


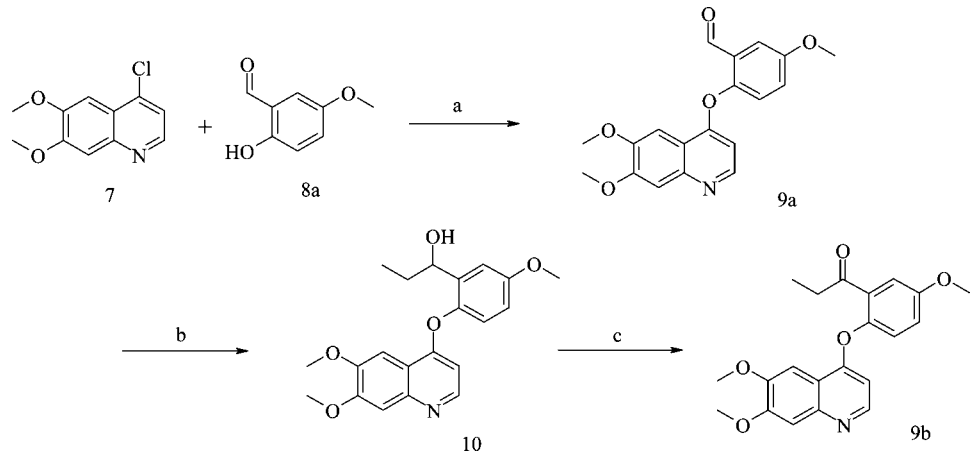
Figure 1. Quinoline-derived kinase inhibitors.

Scheme 1. Synthesis of Phenoxyquinoline Derivatives[a]



[a] Reagents and conditions: (a) 4-dimethylaminopyridine, *o*-dichlorobenzene, 130−180 °C.

Scheme 2. Synthesis of Phenoxyquinoline Derivatives[a]

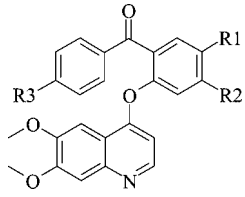


[a] Reagents and conditions: (a) 4-dimethylaminopyridine, chlorobenzene, 130 °C; (b) EtMgBr, THF, −78 °C; (c) *N-tert*-butylbenzenesulfinimidoyl chloride, DBU, CH_2Cl_2, −78 °C, then 0 °C.

Table 3. We demonstrated that the methyl group at R1 is crucial for the activity with acetophenone derivatives (**9r**). Large functional groups are not permitted at R1 (**9v**, **9x**, and **9y**). R2 must be no bigger than the methyl group, and even the methoxy analogues, **9aa** and **9ad**, showed decreased activity. Interestingly, with regard to the acetophenone derivatives, the compound with the methoxy group introduced at R1, which significantly decreased the activity of the benzophenone derivatives, retained its activity (**9w**). Among these acetophenone analogues, the compounds with small substituents at R1 and R2 showed the best potency (**9ab** and **9ac**). Combined with the SARs of the phenylpropanone derivatives (**9b**, **9m**, and **9ae**), these observations shed light on the size of the binding pocket and suggested that the whole ketone moiety should fit within it.

Some of the representative compounds were tested for selectivity against the c-Src kinase (Table 4). Generally, the acetophenone derivatives showed higher selectivity than the benzophenone derivatives. The methyl group at the R1 position increased the selectivity against the c-Src kinase. This observation was supported by the preliminary computational docking model, which indicated that the methyl group occupied the hydrophobic pocket and that a small alkyl group at this position contributed to the activity and the selectivity.

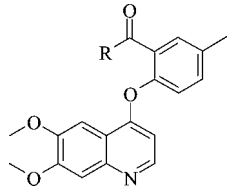
Table 1. SARs of Benzophenone Derivatives



compd	R1	R2	R3	Smad2/3 reporter assay, IC_{50} (μM)
6	H	H	H	4.7
9c[a]	Me	H	H	0.64
9d	Cl	H	H	2.7
9e	Br	H	H	3.2
9f	MeO	H	H	>10
9g	H	MeO	H	4.9
9h[a]	Me	Me	H	1.4
9i	Cl	Me	H	2.1
9j	Me	H	^{t}Bu	8.5

[a] Hydrochloride salt was used.

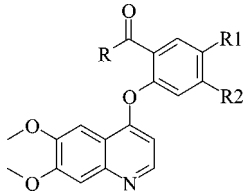
Table 2. SARs of Acyl Derivatives



compd	R	Smad2/3 reporter assay, IC_{50} (μM)
9c[a]	Ph	0.64
9k	H	>10
9l	Me	2.3
9m	Et	0.62
9n	*c*-Pen	2.2
9o	1-piperidinyl	>10
9p	2-furyl	2.7
9q	5-isooxazoyl	2.5

[a] Hydrochloride salt was used.

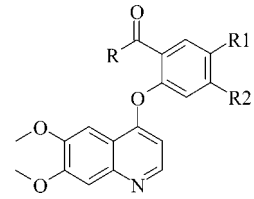
Table 3. SARs of Acetophenone Derivatives



compd	R	R1	R2	Smad2/3 reporter assay, IC_{50} (μM)
9l	Me	Me	H	2.3
9r	Me	H	H	>10
9s	Me	F	H	3.8
9t	Me	Et	H	0.83
9u	Me	Pr	H	0.54
9v	Me	Bu	H	2.2
9w	Me	MeO	H	0.59
9x	Me	EtO	H	5.6
9y	Me	PrO	H	>10
9z	Me	H	Me	5.5
9aa	Me	H	MeO	8.8
9ab	Me	Cl	Me	0.42
9ac	Me	Me	Me	0.37
9ad	Me	MeO	MeO	>10
9m	Et	Me	H	0.62
9b	Et	MeO	H	1.1
9ae	Et	H	MeO	1.8

Table 4 also shows the results of the ALK5 kinase inhibition assay, which were consistent with those of the Smad2/3 reporter assay. These observations supported the notion that the compounds in this series possessed similar cell permeability.

Table 4. Selectivity Profile and ALK5 Kinase Inhibition of Representative Compounds



				IC_{50} (μM)		
compd	R	R1	R2	Smad2/3 reporter assay	c-Src kinase	ALK5 kinase
6	Ph	H	H	4.7	4.5	16
9c[a]	Ph	Me	H	0.64	1.7	2.7
9l	Me	Me	H	2.3	8.4	4.4
9ac	Me	Me	Me	0.37	13	0.63

[a] Hydrochloride salt was used.

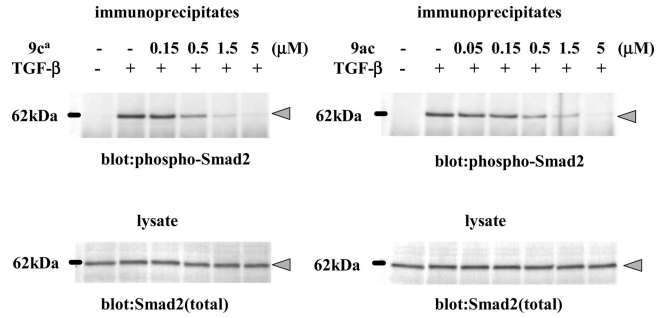


Figure 2. Inhibition of Smad2 phosphorylation. Footnote "a" indicates that the hydrochloride salt was used.

To confirm that the series of compounds inhibited TGF-β signaling, we examined their effects against Smad2 phosphorylation by TGF-β in vitro using cultured cells. Smad2 or Smad3, which is rapidly phosphorylated by TGF-β RI bound to TGF-β, is known to function as a signal transducer of TGF-β signaling. Our results showed that **9c** and **9ac** inhibited Smad2 phosphorylation by TGF-β in a dose-dependent manner (Figure 2). The inhibitory activity (IC_{50}) against Smad2 phosphorylation was around 0.5 μM, which was similar to the values seen in the reporter assay. Because we observed rapid cellular events 30 min after TGF-β stimulation, these results also support the hypothesis that both of the compounds directly interacted with TGF-β RI or TGF-β RII.

Conclusion

We discovered a novel series of TGF-β signaling inhibitors, the members of which appeared to inhibit the TGF-β RI kinase in an ATP-competitive manner. Using the unique hydrophobic pocket of the TGF-β RI kinase domain, we found that the compounds in this series showed good inhibitory activity and good selectivity against the c-Src kinase when appropriate substituents were attached to the phenyl ring. The representative compounds **9c** and **9ac** inhibited Smad2 phosphorylation by TGF-β in a dose-dependent manner. These compounds are expected to show therapeutic efficacy against the diseases areas that are reported to correlate with abnormality of TGF-β signaling.

Experimental Section

Smad2/3 Reporter Assay. The TGF-β inhibitory activity of the compounds was evaluated according to the method previously described.[13] A luciferase reporter plasmid (p(SBE)4-Luc) driven by a promoter comprising four tandem binding sequences of

Smad2/3 was used to detect TGF-β signaling. The reporter plasmid was transduced into human lung cancer epithelial cells (A549, American type Culture Collection [ATCC]) to establish a stable cell line expressing the reporter gene. A test compound and TGF-β1 (2 ng/ml) were added to the cells, and the mixture was cultured for 4 h. The luciferase activity of the cells was then measured by a chemiluminescence method (Steady Glo luciferase assay system, Promega).

{2-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(phenyl)methanone (6). **7** (58 mg), 2-hydroxybenzophenone (271 mg), and 4-dimethylaminopyridine (166 mg) were mixed in *o*-dichlorobenzene (5 mL) and then stirred at 160 °C for 4 h. After cooling to room temperature, the mixture was concentrated. Chloroform was added, and the solution was washed with 1 N NaOH solution and brine and then dried over sodium sulfate. The solution was concentrated, and the residue was purified by column chromatography (elution with chloroform, followed by acetone/hexanes) to give **6** (100 mg, quantitative yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 (s, 3H), 3.99 (s, 3H), 6.43 (d, J = 5.1 Hz, 1H), 6.93 (s, 1H), 7.26 (d, J = 8.3 Hz, 1H), 7.28−7.34 (m, 3H), 7.39−7.48 (m, 2H), 7.59−7.72 (m, 4H), 8.44 (d, J = 5.4 Hz, 1H); MS (ESI) m/z 386 $(M + 1)^+$; purity 98% (method D, t_R = 7.94 min), 100% (method I, t_R = 9.74 min).

1-{2-[(6,7-Dimethoxy-4-quinolyl)oxy]-5-methoxyphenyl}-1-propanone (9b). **7** (2.23 g), **8a** (6.08 g), and 4-dimethylaminopyridine (4.88 g) were mixed in chlorobenzene (40 mL) and then stirred at 130 °C overnight. After the mixture was cooled to room temperature, H_2O was added. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine and then dried over sodium sulfate. The solution was concentrated, and the residue was purified by column chromatography (elution with AcOEt/hexanes) to give **9a** (2.72 g, yield 80%). **9a** (140 mg) was dissolved in tetrahydrofuran (2 mL), and the solution was cooled to −78 °C. A solution of 0.96 M ethylmagnesium bromide in tetrahydrofuran (0.7 mL) was slowly added to the solution. After the mixture was stirred at −78 °C for 1 h, an aqueous solution of ammonium chloride was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine and then dried over magnesium sulfate. The solution was concentrated, and the residue was purified by column chromatography (elution with acetone/hexanes) to give **10** (75 mg, yield 49%). **10** (66 mg) was dissolved in dichloromethane (2 mL), and 1,8-diazabicyclo[5.4.0]-7-undecene (72 mg) was added. The mixture was cooled to −78 °C, and a solution of *N*-*tert*-butylbenzenesulfinimidoyl chloride (71 mg) in dichloromethane was added. The mixture was stirred at −78 °C for 30 min and then at 0 °C for 30 min. H_2O was added, and the mixture was extracted with chloroform. The organic layer was washed with H_2O and then dried over sodium sulfate. The solution was concentrated, and the residue was purified by column chromatography (elution with acetone/hexanes) to give **9b** (56 mg, yield 86%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (t, J = 7.3 Hz, 3H), 2.88 (q, J = 7.3 Hz, 2H), 3.89 (s, 3H), 4.06 (s, 3H), 4.06 (s, 3H), 6.39 (d, J = 5.1 Hz, 1H), 7.07−7.15 (m, 2H), 7.39 (d, J = 2.2 Hz, 1H), 7.45 (s, 1H), 7.57 (s, 1H), 8.49 (d, J = 5.1 Hz, 1H); MS (ESI) m/z 368 $(M + 1)^+$; purity 99% (method D, t_R = 6.71 min), 98% (method I, t_R = 8.23 min).

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Supporting Information Available: Details on general experimental methods, biological assays, the synthesis of **9c**−**ae**, and their analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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