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Bioorganic & Medicinal Chemistry Letters 17 (2007) 2134–2138
Bioorganic & Medicinal Chemistry Letters

Synthesis and activity of quinolinyl-methylene-thiazolinones as potent and selective cyclin-dependent kinase 1 inhibitors

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Received 11 January 2007; accepted 29 January 2007
Available online 31 January 2007

Abstract—A novel series of quinolinyl-methylene-thiazolinones has been identified as potent and selective cyclin-dependent kinase 1 (CDK1) inhibitors. Their synthesis and structure activity relationships (SAR) are described. Representative compounds from this class reversibly inhibit CDK1 activity in vitro, and block cell cycle progression in human tumor cell lines, suggesting a potential use as antitumor agents.

Cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases that play critical roles in controlling cell cycle progression.[1,2] After binding to a family of regulatory proteins cyclins that expressed at different times during the cell cycle, CDKs are activated. Additional control over CDK activity is exerted by phosphorylation on specific threonine residues. Abnormal CDK control of the cell cycle has been linked to the molecular pathology of cancer, and thus inhibitors of cyclin-dependent kinases are anticipated to have antiproliferative activity and potential therapeutic utility. Therefore, CDKs have become attractive therapeutic targets for cancer therapy.[3–5]

It has been established that three CDKs (CDK1, CDK2, and CDK4) and their activating cyclin partners (A, B, D, and E) play key roles in mammalian cell cycle regulation.[1] CDK2/cyclin E and CDK4/cyclin D control passage through the G1-phase and G1- to S-phase transition by phosphorylation of the retinoblastoma phosphoprotein, pRB. CDK2/cyclin A regulates passage through the S-phase. CDK1/cyclin B controls the G2 checkpoint and regulates entry into mitosis. However, it was found that CDK2 and CDK4 may not be essential for cell cycle progression in recent genetic and RNA interference studies.[6–10] Similar experiments with CDK1 have shown that its kinase activity is critical for cell cycle progression through mitosis. Therefore, CDK1 inhibitors are expected to effectively arrest tumor cell growth.

Many small-molecule CDK inhibitors have been identified[11–13] and some of them have entered clinical evaluation for the treatment of cancer.[14–19] Although specific CDK2 and CDK4 inhibitors have been reported, the identification of CDK1-selective small-molecule inhibitors has been challenging. Recently, we discovered a quinolinyl-methylene-thiazolinone as potent and selective CDK1 inhibitor.[20] Here, we report the synthesis, structure activity relationships (SAR), and anti-proliferative activities of the quinolinyl-methylene-thiazolinone class of CDK1 inhibitors.

Most of the quinolinyl-methylene-thiazolinone analogs described in this paper (see Table 1) were prepared by the two general synthetic methods outlined in Scheme 1. In method A, rhodanine was reacted with the appropriate amine **1** in the presence of $HgCl_2$ and DIEA to give the intermediate **3**. Knoevenagel condensation of **3** with the appropriate aldehydes catalyzed by benzoic acid and piperidine in toluene gave the desired products **4a**. In method B, condensation of rhodanine with the appropriate ketone or aldehyde such as 6-quinoline-carboxaldehyde yielded intermediate **6**. Although different Knoevenagel condensation conditions can be used,

Keywords: Cyclin-dependent kinase; CDK1 inhibitor; Anticancer.
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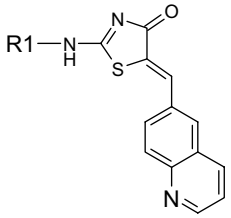
doi:10.1016/j.bmcl.2007.01.081

Table 1. Structure–activity relationship of quinolinyl-methylene-thiazolin-ones against CDK1/cyclinB[a]



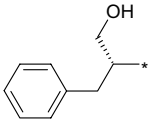
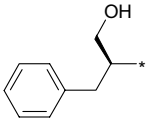
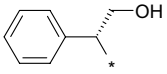
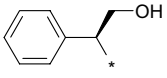
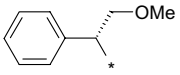
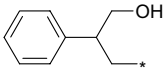
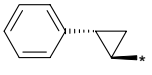
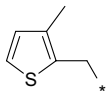
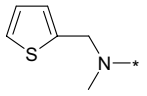
Compound	Rl	K_i (μM)
12	H	0.080
20	MeO	>2.0
21	Me	0.27
22	*n*-Bu	0.31
23	*cyc*-Propyl	0.27
24	Ph	>2.0
25	$PhCH_2$	0.15
26	$PhCH_2CH_2$	0.060
27	$PhCH_2CH_2CH_2$	0.24
28	2-Thienyl-CH_2	0.035
29	2-Thienyl-CH_2CH_2	0.045
30	2-Pyridyl-CH_2CH_2	0.28
31	2-Pyridyl-CH_2	0.31
32	*o*-F-Ph-CH_2CH_2	0.12
33	*m*-F-Ph-CH_2CH_2	0.060
34	*p*-F-Ph-CH_2CH_2	0.062
35	*p*-Cl-Ph-CH_2CH_2	0.41
36	*p*-Br-Ph-CH_2CH_2	0.64
37	*p*-MeOPh-CH_2CH_2	0.39
38	*m*-Cl-Ph-CH_2CH_2	0.13
39	*o*-MeOPh-CH_2CH_2	0.063
40	*o*-EtO-Ph-CH_2CH_2	0.087
41	*o*-Cl-Ph-CH_2CH_2	0.046
42	*o*-Cl-Ph-CH_2	0.052
43	*o*-Br-Ph-CH_2	0.038
44	*o*-MeO-Ph-CH_2	0.086
45	2,6-Di-Cl-Ph-CH_2	1.04
46		0.23
47		2.0
48		0.35
49		0.58
50		>2.0
51		0.39
52		0.023

Table 1 (*continued*)

Compound	Rl	K_i (μM)
53		0.089
54		>2.0

[a] See Ref. 20 for a description of the CDK1 assay.

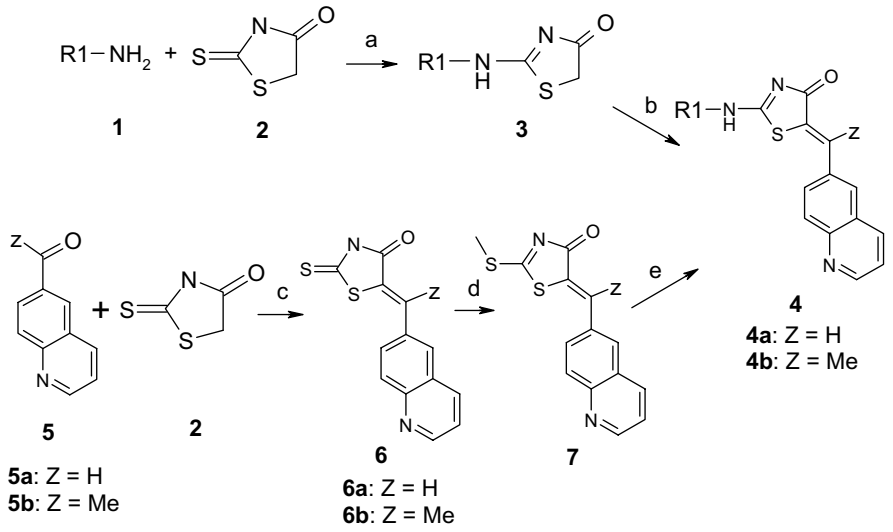
NaOAc/HOAc gave the best result with an excellent yield for **6a** in the case of aldehydes (Z = H). In the case of ketones (Z = Me), a variety of Knoevenagel condensation conditions such as NaOAc/HOAc, NH_4OAc/DMF, and PhCOOH/Piperidine/PhMe were investigated but produced low yields of the product **6b**. However, NH_4OAc in toluene gave an almost quantitative yield of **6b**. Methylation of **6** with MeI provided the thioether intermediate **7**. Subsequent introduction of the R1 group was achieved by treatment of **7** with a variety of amines (R1-NH_2) to give thiazolinone product **4**. In this step, MeCN was the solvent of choice although other solvents such as EtOH and DMF could also be used. Most of the products precipitated at the end of the reaction when MeCN was used as solvent and were isolated by filtration.

Oxazolinone analogs were prepared as shown in Scheme 2 using a procedure similar to method B described above starting from thioxo-oxazolidinone **8** prepared from sodium cyanide, potassium thiocyanate, and formaldehyde. Knoevenagel condensation and methylation of **8** provided the intermediate **10**, which was finally treated with amine to give the product **11**.

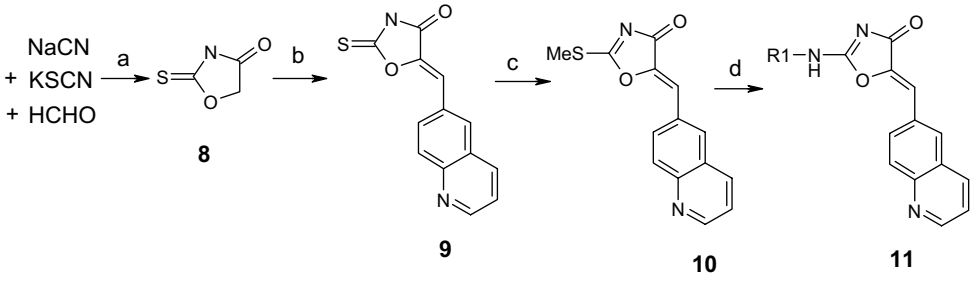
Compounds **12**, **13**, **14**, and **15** were simply prepared as shown in Scheme 3 by Knoevenagel condensation conducted using sodium acetate in acetic acid.

To obtain the analog **19** with a single bond linker between thiazolinone and quinoline rings for activity comparison, direct reduction of **28** under reduction conditions such as hydrogenation, reduction with $LiBH_4$ or Hantzsch ester was tried and proved to be unsuccessful. It was finally prepared using the route shown in Scheme 4 starting from aminoquinoline **17**. Diazotization of **17** yielded diazonium salt intermediate, which was reacted with acrylate in the presence of catalytic CuBr to produce beta-bromo ester **18**. Compound **18** was reacted with a thiourea **16**, prepared from the corresponding amine, to form the thiazolinone ring in the desired product **19**.

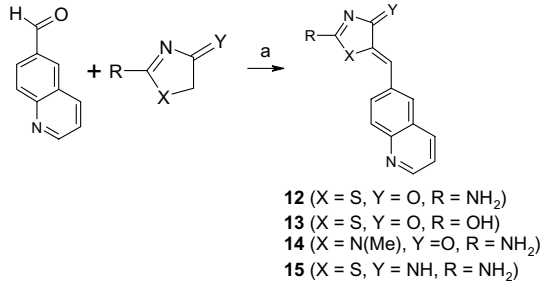
The quinolinyl-methylene-thiazolinones were initially identified by their anti-proliferative activity in a cell-based screen. A distinct block in G2 phase of the cell cycle suggested that CDK1 inhibition might be involved. Their CDK1 inhibitory activity was confirmed by testing for direct inhibition of CDK1/cyclin B activity in vitro.[20] The good correlation between CDK1 inhibitory activity



Scheme 1. General synthetic scheme for quinolinyl-methylene-thiazolin-ones. Reagents and conditions: (a) $HgCl_2$, DIEA, MeCN, rt, 12 h; (b) 6-quinolinecarboxaldehyde, PhCOOH/piperidine, toluene, 150 °C, micro-wave, 20 min (Z = H only); (c) NaOAc, HOAc, 130 °C, 24 h (Z = H); or NH_4OAc, PhMe, 130 °C, 24 h, (Z = Me); (d) MeI, DIEA, EtOH, rt, 12 h; (e) R1-NH_2, DIEA, MeCN, microwave, 145 °C, 20 min.



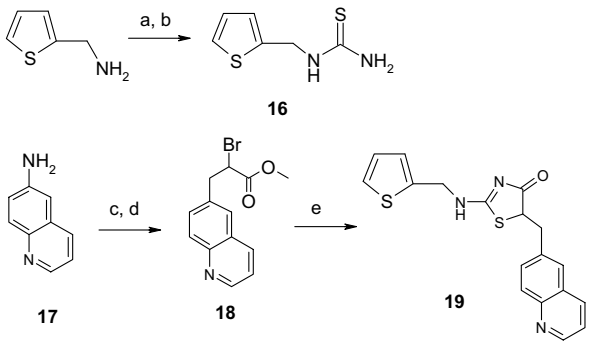
Scheme 2. Synthesis of oxazolinone **11**. Reagents and conditions: (a) concd HCl, H_2O, 0 °C, 30 min, then rt, 12 h; (b) 6-quinolinecarbox-aldehyde, NaOAc, HOAc, reflux, 12 h; (c) MeI, DIEA, EtOH, rt, 12 h; (d) R1-NH_2, microwave, 100 °C, 10 min.



Scheme 3. Synthesis of compounds **12**–**15**. Reagents and conditions: (a) NaOAc, HOAc, 130 °C, 12 h.

and anti-proliferative activity against tumor cells further strengthens the notion that growth arrest is derived from CDK1 inhibition (data not shown).

We next generated a series of analogs of structure **4a** and explored the SAR in their CDK1 activity. The data (Table 1) indicated that R1 can tolerate a variety of changes. The unsubstituted compound (**12**) has a K_i of 80 nM, while alkoxy substitution (**20**) resulted in the loss of activity. Alkyl, cycloalkyl, aryl, and heteroaryl substitution in this position is tolerated. Methylation of the amine resulted in the complete loss of the CDK1 activity (**54** vs. **28**). Synthetic intermediate **7** was also inactive. These results indicated that a free NH group, a hydrogen bond donor, is essential for CDK1 inhibitory activity in this position. The length of the spacer between the aryl and amine groups also affected the activity dramatically. Except for compound **24** which lacks a spacer, several carbon spacers are tolerated, and 1–2 carbon



Scheme 4. Synthesis of compound **19**. Reagents and conditions: (a) PhCO-NCS, $CHCl_3$, reflux, 2 h; (b) aq. Na_2CO_3, 70 °C, 1 h; (c) HBr, $NaNO_2$, Me_2CO, H_2O, 0 °C, 30 min; (d) Me acrylate, cat CuBr, 60 °C, 1 h; (e) **16**, NaOAc, 2-methoxyethanol, 100 °C, 5 h.

length gave the best result (**24** vs. **25**, **26**, **27** or **28** vs. **29**, **30** vs. **31**). With right spacer, most aryl is tolerated, but does not contribute significantly to potency.

We then focused on the 1–2 carbon linker analogs. Most substitutions including halogen and alkoxy groups at the *o*- or *m*-positions of the phenyl ring improved or retained the potency (**32**, **33**, **38**–**44**). *p*-Substitution (**35**–**37**) decreased the activity except in the case of very small groups such as fluorine (**34**). Di-substitution (**45**) resulted in reduced activity. The thiophenyl analog (**28**) with heteroaryl substitution was most potent against CDK1 (K_i = 35 nM).

In the case of phenyl (or heteroaromatic) alkyl (1–2 carbons) as an R1 substituent, we further investigated changes at the C-α or C-β positions. Appropriate substitution at these positions was tolerated. The hydroxylmethyl group was introduced to improve solubility but resulted in a decrease in activity compared with the unsubstituted analogs (**46**–**51**). Stereochemistry also affects the potency. The S-isomer was more potent than the R-isomer (**46** vs. **47** and **48** vs. **49**). The most potent compound in this series with K_i of 23 nM was the more rigid analog (**52**). Since R1 can tolerate many changes, this is a good position to modify the molecule to improve the physicochemical properties.

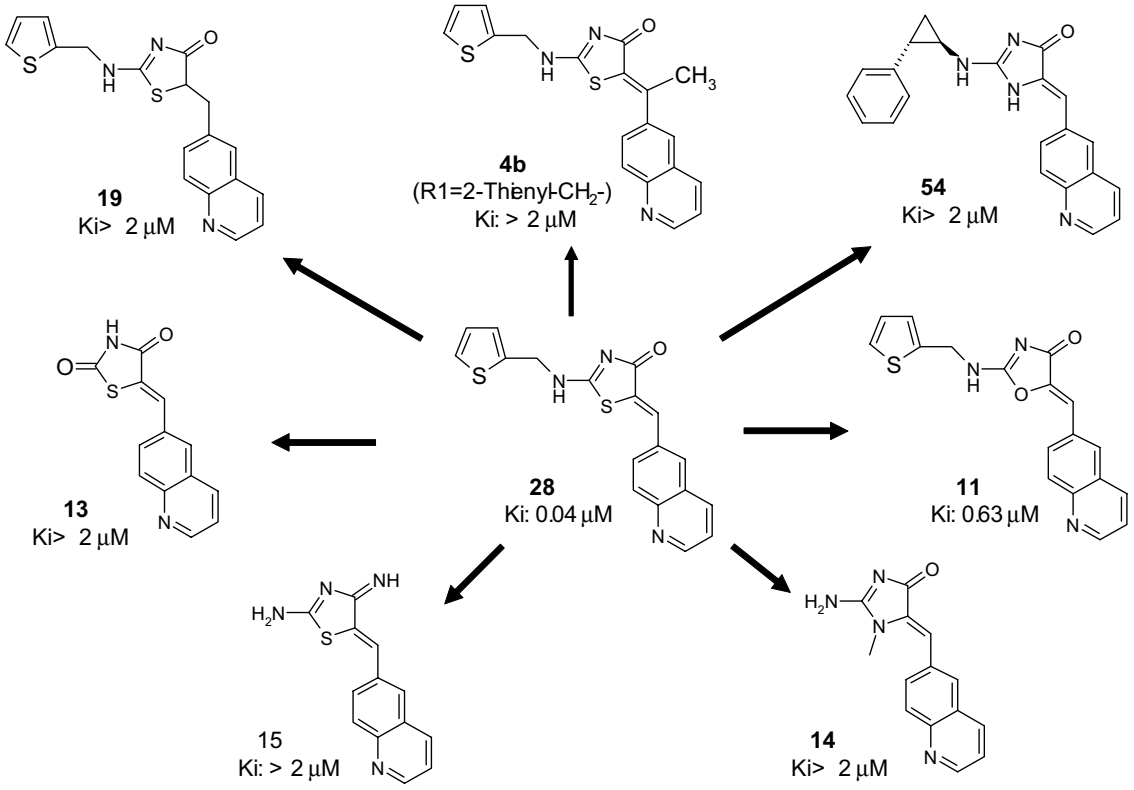


Figure 1. Structure and their CDK1 activities.

Table 2. Selectivity profile against a panel of diverse kinases (K_i, μM)[a]

Compound	CDK1	CDK2	CDK4	PKAP1	AKT	PKCa	PKCd	ERK	GSKp1	FYN	EPHB3
12	0.15	2	>2	>2	>2	>2	1.7	>2	>2	>2	>2
28	0.035	0.34	>2	>2	>2	>2	0.32	2	0.5	>2	>2
52	0.02	>2	>2	>2	>2	>2	0.48	0.22	>2	>2	>2

[a] See Ref. 20 for a description of the kinase assays.

Table 3. Inhibitory activity on various tumor cells for representative compounds[a]

Compound	Tumor cell (IC50, μM)					
	HCT116	H460a	SW480	MDA435	SJSA1	RKO
12	2.53	1.82	3.10	1.28	2.84	1.86
28	1.14	1.04	2.20	1.14	4.55	2.00
46	1.58	ND	3.07	2.03	3.24	1.39
52	2.41	2.49	6.58	3.34	1.85	2.34

[a] See Ref. 20 for a description of the cellular anti-proliferation assays.

The possibility of replacement of the thiazolinone moiety (Fig. 1, **11**, **13**–**15**) was also investigated. However, any attempt to replace it with other heterocyclics was unsuccessful. Only oxazolinone retained some potency.

Next, we examined the linker between the thiazolinone and quinoline rings and found that the double bond is required for activity. Replacement of the double bond with a single bond resulted in a total loss of activity (**19**). A similar result was obtained with the methyl substitution of the hydrogen at the olefin position (**4b**). These results suggested that the unsubstituted olefin linker is important for maintaining the configuration of the thiazolinone and quinoline rings, needed for CDK1 binding.

The selectivity of the most potent CDK1 inhibitors was examined against a panel of kinases including CDK2 and CDK4 (Table 2). CDK1 and CDK2 proteins share a 66% identity and 84% similarity.[21] Modeling studies have shown that the ATP-binding sites of these two kinases are very similar.[22] There are only two differences in their amino acid composition, at positions 84 and 85, and the side chains at these positions project outside of the ATP binding pocket. Nevertheless, compounds **12**, **28**, and **52** show at least 10-fold selectivity for CDK1. The lack of CDK1 crystal structure makes it difficult to explain the structural basis for this selectivity. These compounds also showed at least 10-fold selectivity against all other tested human kinases (Table 2).

Four of the most potent compounds **12**, **28**, **46**, and **52** were selected for evaluation of their in vitro cellular activity against a panel of human cancer cell lines: colon cancer (HCT116, SW480, RKO), lung (H460a), breast (MDA435), and osteosarcoma (SJSA1). They showed antiproliferative activity in all cell lines in the range of 1–7 μM IC50 (Table 3). We reported previously that the antiproliferative activity of **28** is due to a reversible block of cell cycle progression at the G2/M phase border.[20] However, prolonged exposure to the compound leads to induction of apoptosis and suggests that selective CDK1 inhibitors may have utility as anticancer agents.[20]

In conclusion, we have identified the quinolinyl-methylene-thiazolinone class as potent and selective CDK1 inhibitors. SAR studies indicated that the quinoline and thiazoline moieties are important for binding, while the amine position can tolerate more modifications. The antiproliferative and pro-apoptotic activity of these compounds suggests that members of this class could be developed as agents for cancer therapy.

Acknowledgments

We thank Drs. Jefferson Tilley and Adrian Cheung for critically reading the manuscript, and Drs. Nader Fotouhi and John Roberts for the helpful discussions during this work.

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