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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2311–2315
Bioorganic & Medicinal Chemistry Letters

Investigation of novel 7,8-disubstituted-5,10-dihydro-dibenzo-[*b*,*e*][1,4]diazepin-11-ones as potent Chk1 inhibitors

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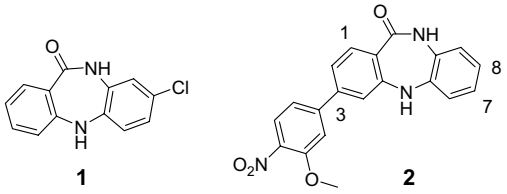
Received 24 January 2008; revised 28 February 2008; accepted 29 February 2008
Available online 6 March 2008

Abstract—The synthesis and structure–activity relationships (SAR) of Chk1 inhibitors based on a 5,10-dihydro-dibenzo[*b*,*e*][1,4]diazepin-11-one core are described. Specifically, an exploration of the 7 and 8 positions on this previously disclosed core afforded compounds with improved enzymatic and cellular potency.

Progression of cells through the cell-cycle is mediated by checkpoints where the integrity of the cell is monitored.[1] Specifically, checkpoint kinase 1 (Chk1 kinase) arrests p-53 deficient tumor cells that have sustained DNA damage due to radiation or chemotherapeutic agents at the G2/M checkpoint.[2,3] This prevents them from entering mitosis and allows time for repair.[4] It is thought that inhibition of Chk1 kinase will lead to abrogation of the G2/M checkpoint, therefore allowing DNA-damaged cells to enter mitosis. This would result in cell death, thus preventing the replication of aberrant cells and the progression of cancer.[3] Since normal cells arrest at the G1/S checkpoint and should remain unaffected, small molecule Chk1 inhibitors have consequently been targeted as possible adjuvant therapies to traditional cancer treatments.[2,5]

In a previous report from our laboratories, we disclosed the identification and subsequent optimization of benzodiazepinone-based HTS hit **1**. In that effort, position 3 was targeted. The X-ray structure of **1** bound to the Chk1 enzyme showed a deep cleft that could accommodate additional functionality at that site. A variety of 3-substituted compounds were thus investigated, leading to the identification of compound **2** as the most potent Chk1 inhibitor within this class.[6] Despite the favorable enzymatic activity, however, compound **2** did not significantly potentiate the activity of the chemotherapeutic agent doxorubicin during co-administration in an MTS assay measuring antiproliferative ability.[6,7] A survey of the remaining sites of the molecule demonstrated that, with the exception of positions 7 and 8, the Chk1 enzyme was intolerant to further substitution of the benzodiazepinone core.[6] Herein we disclose the SAR of 7,8-disubstituted benzodiazepinones and detail the identification of a compound with good enzymatic and cellular profiles.



IC_{50} = 10 nM
MTS EC_{50} = 47.7 μM
MTS EC_{50} with Doxorubicin = 33.8 μM

We began our investigation by substituting position 7 with a number of ether- and carbon-linked moieties while holding position 8 constant as a methoxy group. Substitution at position 8 with a methoxy group had previously given a compound with reasonable enzymatic activity (IC_{50} = 14 nM).[6]

Synthesis of the 7-position ether-substituted benzodiazepinones is shown in Scheme 1. SEM-protection of the

Keyword: Chk1.
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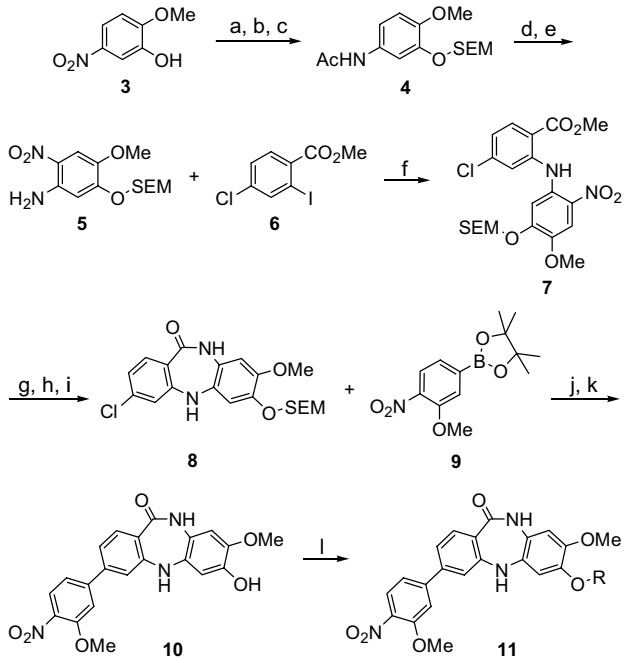
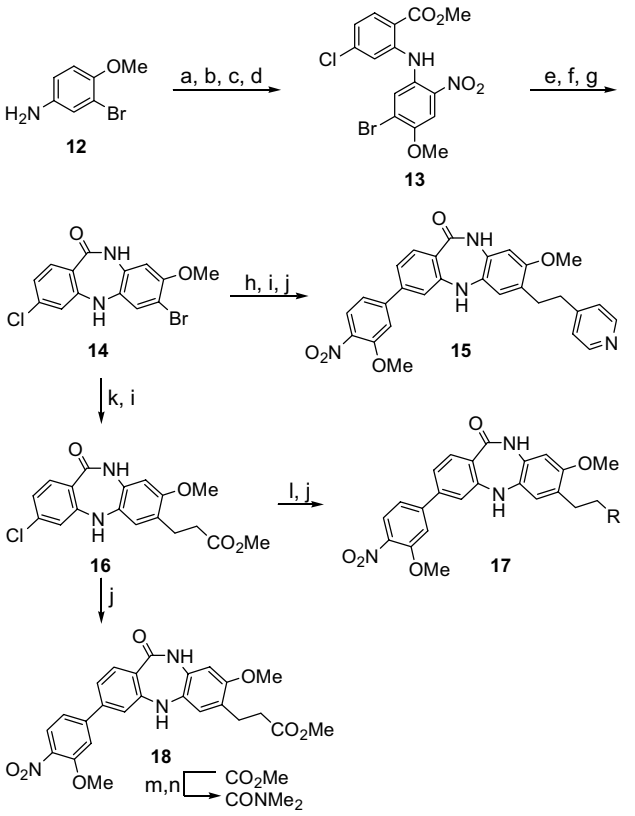
doi:10.1016/j.bmcl.2008.02.080



Scheme 1. Reagents and conditions: (a) SEMCl ($Me_3SiCH_2CH_2OCH_2Cl$), DIPEA, CH_2Cl_2, rt; (b) 5% Pt/C, H_2, EtOH, rt; (c) Ac_2O, DMAP, DCE, rt, 81% over 3 steps; (d) Ac_2O, conc. HNO_3, −20 °C, 70%; (e) K_2CO_3, MeOH, rt, 91%; (f) Cu, K_2CO_3, PhCl, reflux, 84%; (g) LiOH·H_2O, THF, H_2O, 65 °C; (h) Et_3N, $SnCl_2{\cdot}2H_2O$, 65 °C; (i) HATU, Et_3N, DMF, rt, 89% over 3 steps; (j) $Pd(PPh_3)_4$, 1 M Na_2CO_3, 7:2:3 DME/EtOH/H_2O, 160 °C microwave, 62%; (k) 4 N HCl/dioxane, MeOH, CH_2Cl_2, 100%; (l) RX, K_2CO_3, DMF, 100 °C.

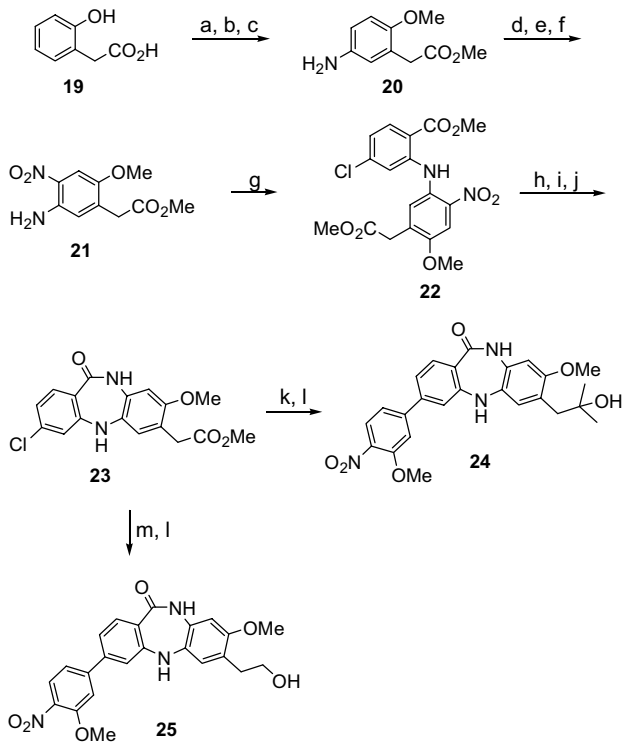


Scheme 2. Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, 0 °C, 95%; (b) Ac_2O, conc. HNO_3, −10 °C, 96%; (c) MeOH, H_2SO_4, reflux, 95%; (d) **6**, Cu, K_2CO_3, PhCl, reflux, 88%; (e) 5% Pt/C, H_2, MeOH, rt, 73%; (f) LiOH·H_2O, THF, H_2O; (g) HATU, Et_3N, DMF, 97% over two steps; (h) 4-pyridyl-CH=CH_2, $Pd(dppf)Cl_2$, Et_3N, DMF, 110 °C; (i) H_2, 5% Pt/C, MeOH, rt, 17% over 2 steps toward **15**, 70% for **16**; (j) **9**, $Pd(OAc)_2$, Cy-Map, CsF, DMF, 85 °C, 29% for **15**, 73% for **18**; (k) MeO_2C–CH=CH_2, $Pd(dppf)Cl_2$, Et_3N, DMF, 110 °C, 82%; (l) LAH (for **17a**, 80%) or MeMgBr (for **17b**, 78%), THF; (m) LiOH·H_2O, DMF, THF, H_2O, 100 °C, 48%; (n) $Me_2NH{\cdot}HCl$, HATU, Et_3N, DMF, rt, 91%.

hydroxyl group of 2-methoxy-5-nitrophenol (**3**) followed by nitro reduction and acetylation of the resulting aniline gave intermediate **4**. Nitration followed by deprotection of the amine afforded **5**. Copper-assisted coupling of compound **5** with methyl ester **6** (obtained by esterification of the commercially available acid) yielded amine **7**. The benzodiazepinone core **8** was formed in a 3-step, one-pot reaction through ester hydrolysis, tin-mediated nitro-group reduction and cyclization via HATU-activated amide formation. Suzuki coupling of intermediates **8** and **9** (prepared from 5-chloro-2-nitroanisole) followed by removal of the SEM protecting group provided **10**. Alkylation of the 7-hydroxy with various alkyl halides afforded the final ether products (**11a**–**j**).

The 7-position carbon-substituted analogs **15**, **17**, and **18** were prepared as shown in Scheme 2. Acetylation of 3-bromo-4-methoxyaniline (**12**) followed by nitration, deacetylation, and copper-mediated coupling with **6** resulted in intermediate **13**. Platinum-catalyzed nitro-group reduction, ester hydrolysis, and intramolecular amide formation gave benzodiazepinone intermediate **14**. The pyridylethyl analog **15** was prepared by a Heck coupling with 4-vinylpyridine and subsequent hydrogenation of the double bond, then attachment of the 3-methoxy-4-nitrophenyl ring via Suzuki coupling. Alternatively, Heck coupling of intermediate **14** with methyl acrylate followed by hydrogenation of the resulting olefin afforded intermediate **16**. Lithium aluminum hydride reduction of ester **16** followed by Suzuki coupling with **9** gave **17a**. Grignard reaction with methylmagnesium bromide and subsequent Suzuki coupling produced **17b**. Arylation of **16** with pinacol borate **9** provided **18a** which was converted to **18b** via ester hydrolysis and HATU-assisted amidation.

Carbon-linked analogs **24** and **25** were synthesized as shown in Scheme 3. Nitration of (2-hydroxyphenyl)acetic acid (**19**) was followed by treatment with TMS-diazomethane resulting in methylation of both the hydroxyl and carboxyl groups. Catalytic hydrogenation of the nitro group afforded aniline **20**. Acetyl protection of the aniline followed by nitration and then deprotection gave **21**. Copper-mediated coupling of **21** with **6** resulted in intermediate **22**. Reduction of the nitro group with subsequent ester hydrolysis and intramolecular amide formation gave benzodiazepinone **23**. Treatment of **23** with methylmagnesium bromide then Suzuki coupling with **9** produced compound **24**. Alternatively, lithium aluminum hydride reduction of the ester of **23** followed by Suzuki coupling provided alcohol **25**.



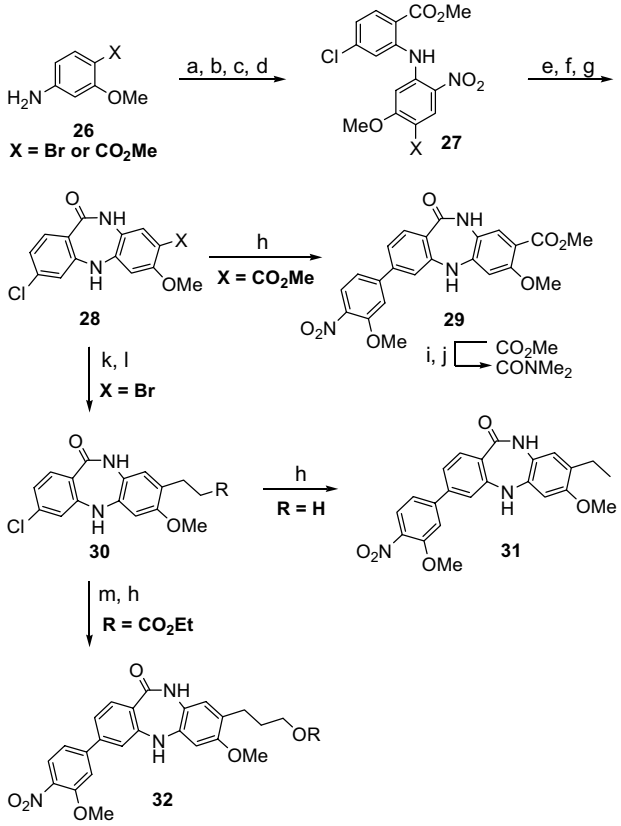
Scheme 3. Reagents and conditions: (a) conc. HNO_3, H_2O, 0 °C; (b) TMS–CHN_2, CH_2Cl_2, MeOH, 45% over two steps; (c) Pd/C, H_2, MeOH; (d) Ac_2O, Et_3N, CH_2Cl_2, 0 °C, 88% over two steps; (e) Ac_2O, conc. HNO_3, −10 °C, 88%; (f) MeOH, H_2SO_4, reflux, 95%; (g) **6**, Cu, K_2CO_3, PhCl, reflux, 80%; (h) 5% Pt/C, H_2, MeOH, rt, 93%; (i) LiOH·H_2O, THF, H_2O; (j) HATU, Et_3N, DMF, 75% over two steps; (k) MeMgBr, THF, 75%; (l) **9**, $Pd(OAc)_2$, Cy-Map, CsF, DMF, 85 °C, 68% for **24**, 75% for **25**; (m) LAH, THF, 80%.

We found that incorporation of polar groups at position 7 provided compounds with greater activity against Chk1 than did hydrophobic groups. Specifically, compounds containing hydroxy and methoxy groups, as well as straight-chain hydroxyalkyl or aminoalkyl ethers had IC_{50} values of <1 nM (Compounds **10**, **11a**–**d**). Varying the length of the substitution from 4–6 atoms did not alter the activity. The hydrophobic 2-trifluorobenzyl ether compound (**11f**) was over 1000-fold less active than the straight-chain ethers. Heteroaryl and aliphatic heterocycles were tolerated, however, they were typically 5- to 10-fold less active than the straight-chain ether-containing compounds (**11g**–**j**). The 7-position carbon-linked analogs were generally less active than the corresponding ether analogs (**11b** vs **17a** and **11h** vs **15**). The addition of geminal dimethyl groups on the terminal carbons of the straight-chain carbon-linked compounds was well tolerated (**17b** vs **17a** and **24** vs **25**). The presence of larger or more hydrophobic substituents conferred deleterious effects on the enzymatic potency (data in Table 1 and data not shown).

Having surveyed substitutions at position 7 of the benzodiazepinone, we proceeded with the analysis of position 8 while position 7 was held constant as methoxy. Our survey of position 8 was limited to carbon substitutions as attempts to produce ethers via either alkylation or Mitsunobu reaction resulted in preferential N-alkylation. Similar transformations to those described previously for Schemes 1–3 allowed for efficient preparation of the carbon-substituted compounds (Scheme 4).

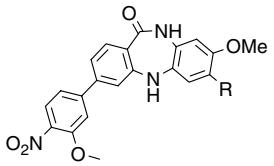
A limited number of compounds with small substituents at position 8 are presented in Table 2. As with our findings for position 7, a hydroxyalkyl substituent proved to be optimal for maximizing inhibitor potency (compound **32**). Methyl ester, dimethylamide, and ethyl substitution (**29a**, **29b** and **31**, respectively) were not well tolerated in comparison to the hydroxypropyl moiety, conferring diminished activity by 5- to 8-fold. It was not surprising that polar substitutions were superior to non-polar substitutions at both positions 7 and 8 since the X-ray structure of our HTS hit compound **1** bound to the Chk1 enzyme had shown that these positions point toward the hydrophilic solvent front.[6]

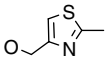
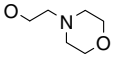
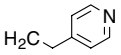
Compounds with enzymatic activities less than 50 nM were taken forward into three cellular assays, MTS (a measure of antiproliferative ability),[6,7] FACS (a mea-



Scheme 4. Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, 0 °C, 100% for X=Br; (b) Ac_2O, conc. HNO_3, −10 °C; (c) K_2CO_3, MeOH, 67% over two steps for X=Br, 91% for X=CO_2Me; (d) **6**, Cu, K_2CO_3, PhCl, reflux, 67% for X=Br, 57% for X=CO_2Me; (e) LiOH·H_2O, THF, MeOH, H_2O, 50 °C; (f) $SnCl_2$·$2H_2O$, 70 °C; (g) HATU, Et_3N, DMF, rt, 65% over three steps for X=Br, 20% over three steps for X=CO_2Me; (h) **9**, $Pd(OAc)_2$, Cy-Map, CsF, DMF, 85 °C, 95% for **29**, 47% for **31**, 60% for **32**; (i) LiOH·H_2O, DMF, THF, H_2O, 100 °C, 27%; (j) Me_2NH·HCl, HATU, Et_3N, DMF, rt, 27%; (k) R–CH=CH_2, $Pd(dppf)Cl_2$, Et_3N, DMF, 110 °C, 66%; (l) H_2, 5% Pt/C, MeOH, 95% for R=H, 94% for R=CO_2Et; (m) LAH, THF, 0 °C, 99%.

Table 1. Inhibition of benzodiazepinones against the Chk1 enzyme

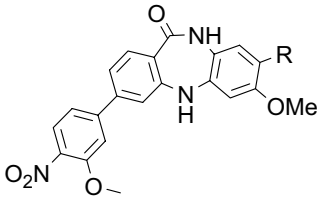


Compound	R	IC_{50} (nM)[7]	MTS (μM) EC_{50}/ EC_{50} with doxorubicin[7]	Facs (μM) EC_{50}/ EC_{50} with doxorubicin[7]	Cdc25A EC_{50} (μM)[8]
10	OH	0.9	>59/2.5	>10/2.3	0.07
11a	OCH_3	0.6	>59/2.7	>10/0.4	0.28
11b	OCH_2CH_2OH	0.58	>59/1.2	>10/1.4	3.1
11c	$OCH_2CH_2CH_2CH_2OH$	0.7	>59/1.0	>10/1.6	2
11d	$OCH_2CH_2CH_2NH_2$	0.98	17.3/2.1	>10/>10	2.6
11e	$OCH_2CH_2OCH_2CH_2OCH_3$	3.1	52/0.8	>10/1.4	3.1
11f		1050	>59/2.4	>10/2.4	NA
11g		4.0	>59/>5.9	>10/>10	>10
11h		6.8	>59/1.7	>10/1.6	0.05
11i		4.8	2.0/0.2	2.4/0.4	0.2
11j		8.1	2.0/0.2	2.4/0.4	0.2
15		16.2	9.6/0.6	>10/0.4	2.1
17a	$CH_2CH_2CH_2OH$	2.6	>59/1.8	>10/0.45	1.5
17b	$CH_2CH_2C(CH_3)_2OH$	4.0	>59/0.6	>10/0.55	0.6
18a	$CH_2CH_2CO_2CH_3$	22	>59/>5.9	>10/>10	>10
18b	$CH_2CH_2CON(CH_3)_2$	7.9	>59/2.6	>10/0.9	0.05
24	$CH_2C(CH_3)_2OH$	3.4	>59/1.2	1.8/0.6	0.04
25	CH_2CH_2OH	3.5	>59/3.0	>10/0.44	0.9

sure of specificity for Chk1 through G2/M checkpoint abrogation)[6,7] and Cdc25A (a measure of Chk1 inhibition through S checkpoint abrogation).[8] We defined an ideal Chk1 inhibitor as having no single agent activity in the MTS and FACS assays (MTS $EC_{50} > 59$ μM and FACS $EC_{50} > 10$ μM), but significant combination activity when co-administered with a DNA-damaging agent ($EC_{50} < 1$ μM). The ideal EC_{50} value for the Cdc25A assay was also defined to be <1 μM.

According to the parameters set forth for an ideal Chk1 inhibitor, only compound **17b** performed as desired in all three cellular assays. Compounds with unbranched hydroxyalkyl substituents (**11b**–**c**, **17a**, and **25**) typically possessed adequate cellular activity. These compounds often showed ideal or near ideal activity in one or more of the cellular assays. Compounds **11b** and **11c** showed similar cellular profiles with no single agent cellular activity in either the MTS or FACS assays, but had combination activities of 1.0–1.6 μM, just above the upper limit for an ideal compound. The similar carbon-linked compounds (**17a** and **25**) also showed no single agent activity, but were better G2/M abrogators with ideal combination FACS EC_{50} values of 0.45 and 0.44 μM, respectively. They failed to meet the requirements of the MTS assay, however, with compound **17a** having slightly diminished antiproliferative activity (MTS $EC_{50} = 1.8$ μM) and compound **25** (MTS $EC_{50} = 3.0$ μM) showing an even greater loss. In contrast, 8-substituted hydroxypropyl analog **32** demonstrated significant single agent activity in both the MTS (single agent $EC_{50} = 22$ μM) and FACS (single agent $EC_{50} = 4.3$ μM) assays. Ester substituents, whether directly attached to the benzodiazepinone core as in compound **29a** or attached through a two-

Table 2. Inhibition of 7-oxygen,8-carbon disubstituted benzodiazepinones against the Chk1 enzyme



Compound	R	IC_{50} (nM)[7]	MTS (μM) EC_{50}/ $EC_{50}$with doxorubicin[7]	Facs (μM) EC_{50}/ EC_{50} with doxorubicin[7]	Cdc25A EC_{50} (μM)[8]
29a	CO_2CH_3	11.2	>59/>5.9	>10/>10	>10
29b	$CON(CH_3)_2$	15.5	45/4.5	>10/>10	3.5
31	CH_2CH_3	9.0	4.5/2.6	>10/1.2	0.35
32	$CH_2CH_2CH_2OH$	1.9	22/1.2	4.3/0.7	0.22

carbon linker as in compound **18a**, showed no activity in any of the three assays. The same results were seen for the 4-pyridylmethoxy substituted compound **11h**. Interestingly, the corresponding carbon analog (**15**) showed significant single agent activity in the MTS assay, ideal activity in the FACS assay and moderate activity in the Cdc25A assay.

Herein we have described the synthesis and SAR of a variety of 7,8-disubstituted-5,10-dihydro-dibenzo[*b*,*e*][1,4]diazepin-11-one compounds. Several of these compounds showed an improved in vitro profile relative to parent compound **2**. Specifically, compound **17b** met all of our in vitro requirements having an IC_{50} of 4.0 nM, combination EC_{50} values with doxorubicin of 0.6 and 0.55 μM in the MTS and FACS assays, respectively, and an EC_{50} value of 0.6 μM in the Cdc25A assay. Thus, substitution of positions 7 and 8 provided improved cellular activity and progress toward obtaining the desired profile.

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