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Synthesis and biological evaluation of novel heterocyclic quinones as inhibitors of the dual specificity protein phosphatase CDC25C

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Abstract—A focused set of heterocyclic quinones based on the benzothiazole, benzoxazole, benzimidazole, indazole and isoindole was prepared and screened with respect to the inhibition of the phosphatase activity of CDC25C. Benzoxazole- and benzothiazole-diones were at least 50 times more potent in inhibiting CDC25C than their benzimidazole-indazole- or isoindole-dione counterparts. These in vitro activities were in good correlation with the anti-proliferative effects observed with Mia PaCa-2 and DU-145 human tumor cell cultures. The IC_{50} values obtained by WST-1 colorimetric assay ranged from 0.10 to 0.50 μM for the benzoxazole- or benzothiazole-diones and were above 10 μM for the other heterocyclic diones. This study further illustrates how the activity of the quinone pharmacophore can be selectively modulated by changing the type of five-membered heterocycle fused to the quinone ring.

The CDC25 dual-specificity phosphatases play an important role in regulating the cell cycle by dephosphorylating and activating CDK/cyclin complexes at key check points, and therefore constitute interesting biological targets for the development of novel anti-proliferative agents.[1–4] For the moment, the subtype of CDC25 that should be targeted remains speculative and the means to obtain selectivity among the various CDC25 isoforms have not been reported. Although we use recombinant CDC25C to screen and optimize our compounds, a pan-CDC25 inhibitory approach is actually pursued. We have identified BN82002 as a new inhibitor of the CDC25 phosphatase activity and observed its characteristic effects at the molecular and cellular levels, as well as in animal models.[5,6] Previously, Lazo and colleagues had reported the potent CDC25 inhibition induced by substituted quinolinediones such as NSC 663284 (**1a**) and other closely related analogues.[7] The bare aza-naphthoquinone moieties of these molecules were insufficient for potent CDC25 inhibition and required an enhancing substituent at position 7 of the quinolinedione template such as 2-morpholin-4-ylethylamino or 1-indan-1-ylamino. These compounds also featured a chloro substituent at the other available site of the quinone ring, position 6 of quinolinedione. Recently, we have identified and characterized BN82685 (**2a**) as thiazole-quinone CDC25 inhibitor which is active in vitro and in vivo.[8] The present letter reports the evaluation as CDC25C phosphatase inhibitors of a focused set of isoesters of **2a**, formed by five-membered heterocycles fused to a benzoquinone (**3**–**6**, Fig. 1). The fused heterocyclic rings consist of three atom bridges which may bear various substituents to broaden structural diversity. The scope of this study is limited to a single *N*,*N*-dimethylethylenediamine side chain on the quinone ring and methyl, ethyl or phenyl substitution at the middle atom of the bridge to form the five-membered heterocyclic ring.

Benzothiazoledione (**10**) was prepared according to the known method[9] with minor modification, as shown in Scheme 1. Commercially available methoxybenzothiazole **7** was nitrated and reduced to give amine **9**, which was then oxidized to the corresponding quinone **10** with Fremy's salt [potassium nitrosodisulfonate, $(KO_3S)_2NO$].

Keywords: Heterocyclic quinone; CDC25 phosphatase; Selectivity; Anti-proliferative effect.
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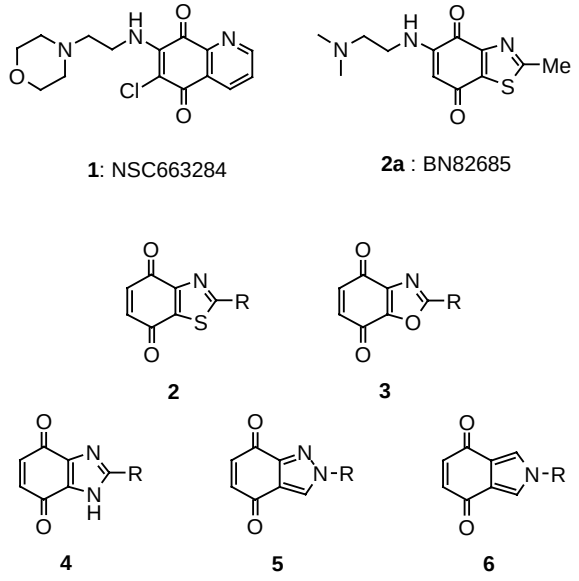


Figure 1.

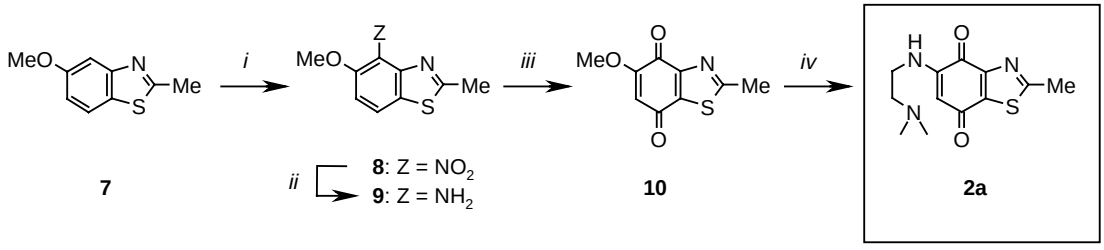
Substitution of the methoxy group with the *N*,*N*-dimethylethylenediamine provided benzothiazoledione **2a**.

The initial route to benzoxazolediones (**3**) is shown in Scheme 2 and consisted of the condensation of 2-amino-3-nitrophenol (**11**) with either carboxylic[10,11] acids or orthoesters[12] to give substituted nitrobenzoxazoles **12**. A subsequent reduction of the nitro group[12] provided aminobenzoxazoles, such as **13**, which were oxidized with either Fremy's salt [potassium nitrosodisulfonate, $(KO_3S)_2NO$][13] or BTI [bis(trifluoroacetoxy)iodobenzene][14] to the corresponding quinone **14**. Addition of *N*,*N*-dimethylethylenediamine to phenylbenzoxalonedione **14** gave a mixture of regioisomers **3b**,**b**′ and **3c**,**c**′. ^{1}H NMR analysis revealed that ethylbenzoxazole **3b**,**b**′ consisted of a 30:1 ratio of two regioisomers as determined by the integration of the N-*H* side-chain signals at 7.39 and 7.22 ppm, while phenylbenzoxazole **3c**,**c**′ consisted of a 1:3.3 mixture of regioisomers from the peaks at 7.49 and 7.33 ppm.

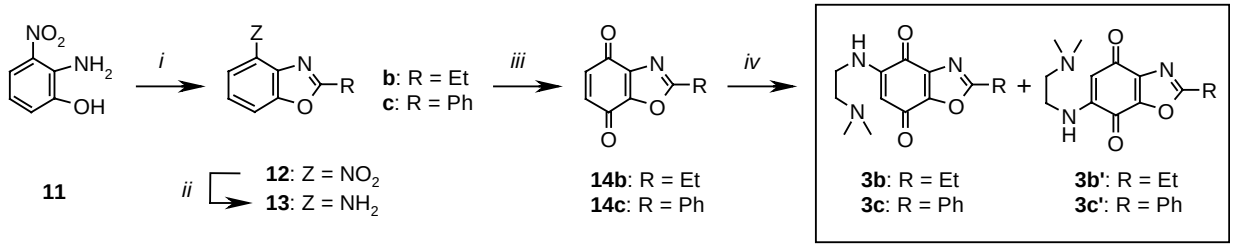
Benzimidazoles were prepared according to previously described procedures,[15,16] from dimethoxybenzene **15** by a nitration reduction sequence to give *ortho*-diaminobenzene **16** (along with its *para*-diamino regioisomer), as shown in Scheme 3. Intermediates were condensed with orthoesters to give dimethoxybenzimidazoles **17**.[17] Benzimidazolediones **18** were obtained by direct oxidation of dimethoxy intermediates with ceric ammonium nitrate [CAN, $Ce(NH_4)_2$ $(NO_3)_6$],[18] and treated with *N*,*N*-dimethylethylenediamine to give the desired test compounds. These products are mixtures of tautomers in rapid equilibrium, represented as **4b**,**b**′ and **4c**,**c**′, respectively. In each case, only a single signal was observed by ^{1}H NMR for the N *H* side-chains at 7.13 and 7.22 ppm, respectively.

Indazolediones **5** were prepared by thermal decomposition of 3-phenylsydnone (**19**)[19] in the presence of *para*-benzoquinone to provide phenylindazoledione (**20**, Scheme 4). Upon treatment with *N*,*N*-dimethylethylenediamine indazolediones **5b**,**b**′ were obtained as a 4.7:1 mixture of regioisomers, determined by the integration of the N-*H* side-chain signals at 7.22 and 7.10 ppm.

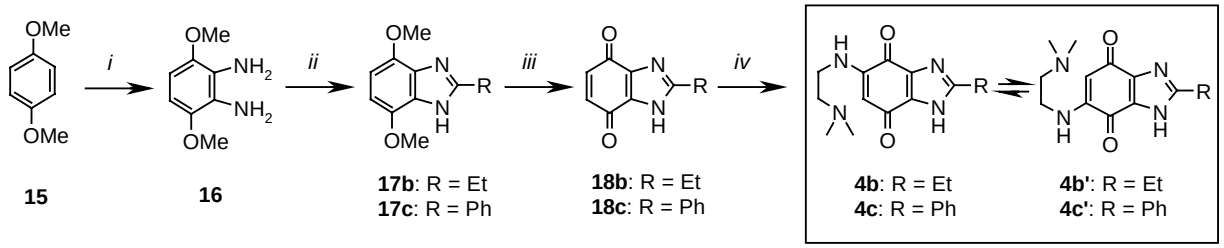
As shown in Scheme 5, isoindolediones were obtained by trapping with *para*-benzoquinone the azomethine ylide generated by heating sarcosine (**21**) in the presence of formaldehyde.[20] The usual treatment with *N*,*N*-dimethylethylenediamine provided isoindoledione **6a**.



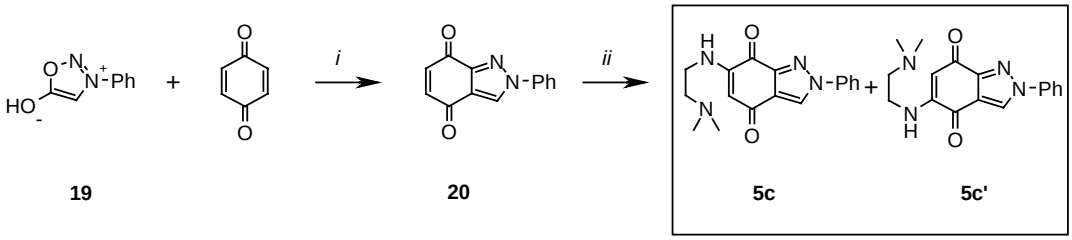
Scheme 1. Reagents and conditions: (i) conc. HNO_3/conc. H_2SO_4, rt, 2 h, 64%; (ii) H_2, Pd/C, EtOH, 94%; (iii) Fremy's salt (1.8 eq), NaH_2PO_4 (0.3 M), acetone, 25 °C, 2 h, 78%; (iv) $Me_2NCH_2CH_2NH_2$ (1.5 eq), MeOH, 60 °C, 2 h, 65%.



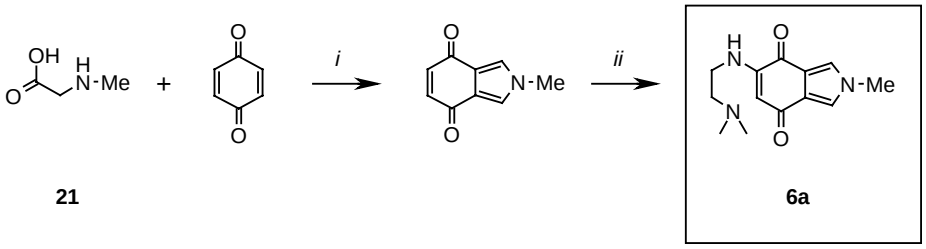
Scheme 2. Reagents and conditions: (i) **12b**: $EtC(OEt)_3$ (1 eq), neat, PTSA (cat), 110 °C, 2 h, 80%; **12c**: $PhC(OMe)_3$ (2 eq), neat, PTSA (cat), 110 °C, 2 h, 82%; (ii) H_2 (8 atm) 10% Pd/C (cat), MeOH, **13b**: 93%, **13c**: 70%; (iii) BTI (2.2 eq), MeCN/H_2O, −5 °C, 30 min, **14b**: 100%, **14c**: 42%; (iv) $Me_2NCH_2CH_2NH_2$ (2 eq), $CeCl_3$–$7H_2O$ (1.1 eq), EtOH, rt 1 h, **3b**,**b**′: 37%, **3c**,**c**′: 10%.



Scheme 3. Reagents and conditions: (i) see Ref. 17 (ii) **17b**: $EtC(OEt)_3$ (2 eq), neat, PTSA (cat), 110 °C, 1.6 h, 61%; **17c**: $PhC(OMe)_3$ (2 eq), neat, PTSA (cat), 110 °C, 1.1 h, 84%; (iii) CAN (4 eq), $AcOEt/H_2O$, −5 °C, 45 min, **18b**: 17%, **14c**: 21%; (iv) $Me_2NCH_2CH_2NH_2$ (1.5 eq), $CeCl_3$-$7H_2O$ (1.1 eq), EtOH, reflux 1 h, **4b**,**b′**: 10%, **4c**,**c′**: 6%.



Scheme 4. Reagents and conditions: (i) *p*-benzoquinone (2 eq) xylene, reflux, 3 h, 24%; (ii) $Me_2NCH_2CH_2NH_2$ (1 eq), EtOH, rt 10 min, 14%.



Scheme 5. Reagents and conditions: (i) *p*-benzoquinone (1 eq), sarcosine (2 eq), paraformaldehyde (5 eq) toluene, reflux (Dean–Stark), 4.5 h, 3%; (ii) $Me_2NCH_2CH_2NH_2$ (1.1 eq), EtOH, rt 10 min, 19%.

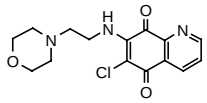
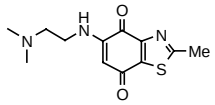
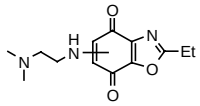
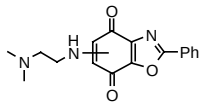
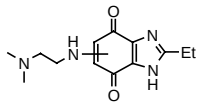
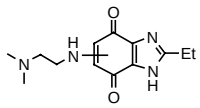
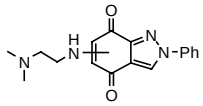
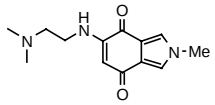
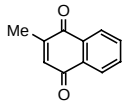
For a preliminary study, test compounds were assayed as mixtures of regiosiomers. Subsequently, we have developed a regioselective method to prepare aminated benzoxazolediones such as **3b** or **3b′** as pure compounds, via pre-established methoxy groups.[21] In other instances, regioselectively aminated heterocyclic quinones have been obtained via pre-established halogen[22] substituents.

The foregoing quinonoid compounds were evaluated for their inhibitory activity against recombinant CDC25C phosphatase, using 3-*O*-methylfluorescein phosphate (OMFP) as a substrate.[6] The results (50% inhibitory concentrations, measured in duplicate) are reported in Table 1. Benzothiazole BN82685 (**2a**) is a potent inhibitor of CDC25C with an IC_{50} value of 0.15 μM, comparable to 0.25 μM obtained with NSC 663284 (**1a**), while menadione used as a benchmark had an IC_{50} of 19 μM. Potent inhibition of CDC25C was also observed for benzoxazolediones **3b**,**b′** and **3c**,**c′**, with ethyl and phenyl substitution, respectively. In contrast, imidazolediones **4b**,**b′** and **4c**,**c′**, indazolediones **5c**,**c′**, and isoindoledione **6a** were found to be much less active, with inhibitory concentrations several orders of magnitude higher than that of benzothiazoledione **2a**.

The set of quinonoid compounds was also assayed for the inhibition of proliferation of two human cancer cell lines, pancreatic carcinoma Mia PaCa-2 and androgen-independent prostate carcinoma DU145. Transient tumor growth inhibition was obtained in nude mice bearing Mia PaCa-2 xenografts,[8] and a recent study by Ozen and Ittmann shows that CDC25C expression and activity are increased in prostate cancer cells, including in DU145.[23] The resulting IC_{50} values were determined with a colorimetric assay based on the cleavage of tetrazolium salt WST1 by mitochondrial dehydrogenase in viable cells and are reported in Table 1. With IC_{50} values ranging from 0.11 to 0.44 μM, good antiproliferative activities were obtained with benzothiazole **2a** as well as benzoxazolediones **3b**,**b′** and **3c**,**c′**. Imidazolediones **4b**,**b′** and **4c**,**c′** were found to be only moderately active with IC_{50} values higher than 10 μM. No effect on cellular proliferation could be detected with indazolediones **5c**,**c′** and isoindoledione **6a**.

Within this focused set of heterocyclic quinones, a good correlation is observed between CDC25C inhibition and antiproliferative activity. As reported recently for

Table 1.

Compound	Structure	CDC25C[a] (μM)	Cell proliferation[b] (μM)	
			Mia PaCa-2	DU145
1		0.25 ± 0.033	2.5 ± 0.095	2.3 ± 0.070
2a		0.15 ± 0.010	0.18 ± 0.034	0.11 ± 0.0060
3b,**3b′**		0.13 ± 0.051	0.44 ± 0.035	0.22 ± 0.016
3c,**3c′**		0.23 ± 0.029	0.15 ± 0.019	0.21 ± 0.0020
4b,**4b′**		12 ± 0.76	18 ± 7.7	>10
4c,**4c′**		17 ± 1.4	12 ± 0.25	>10
5c,**5c′**		12 ± 0.86	ne[d]	ne
6a		81 ± 13	ne	ne
mnd[c]		19 ± 2.9	6.2 ± 0.15	11 ± 0.10

[a] Inhibition of the activity of a maltose binding protein (MBP)-CDC25C recombinant enzyme monitored with 3-*O*-methylfluorescein phosphate (OMFP). The 50% inhibitory concentration mean values ±0 SEM were calculated from at least two, usually three, independent experiments.

[b] The 50% inhibitory concentration mean values ±0 SEM of tumor cell proliferation were determined with tetrazolium salt WST1 by mitochondrial dehydrogenases in viable cell experiment. These experiments were performed at least twice with four or eight determinations per tested concentration.

[c] mnd: menadione.

[d] ne: no effect.

thiazole **2a**,[8] oxazoles **3b**,**b′** and **3c**,**c′** are likely to be pan-CDC25 inhibitors, and their selectivity with respect to other phosphatases is not known. Nevertheless, varring the heterocyclic part of these quinonoids allows us to fine-tune their activity and reveal an interesting aspect of their selectivity. Related molecules which bear the scaffolds of imidazolediones such as **4**,[24–29] indazolediones like **5**,[30,31] or isoindoledione **6**[32] are reported to be active against various biological targets and to have antiproliferative effects, but they do not have the patterns of substitution of the present set and their heterocyclic quinonoid scaffolds alone are not sufficient to exert potent CDC25 inhibition. Conversely, benzothiazoles and benzoxazoles such as **2a**, **3b**,**b′**, and **3c**,**c′** are among the most potent inhibitors of CDC25 phosphatases reported to date and deserve further investigation in the hope of providing new routes to treat cancer patients.

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