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Discovery and structural optimization of pyrazole derivatives as novel inhibitors of Cdc25B

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ABSTRACT

Structural optimization and preliminary structure–activity relationship studies of a series of N-substituted maleimide fused-pyrazole analogues with Cdc25B inhibitory activity, starting from a high-throughput screening hit, are illustrated. A simplified 3,5-diacyl pyrazole analogue was obtained as the most potent compound (**118**, IC_{50} = 0.12 μM) with a 270-fold increase in potency.

Cdc25 phosphatases are key regulators of the cell cycle.[1] Three Cdc25 homologues have been identified in humans—Cdc25A, Cdc25B, and Cdc25C. The overexpression of Cdc25A and Cdc25B has been reported in numerous human tumors,[1–4] and the inhibition of Cdc25 phosphatases may represent a promising therapeutic approach for treatment of cancer.[5,6] So far, a number of small-molecule Cdc25 phosphatase inhibitors have been reported.[7–22]

Among these known series of Cdc25 inhibitors, the most potent inhibitors reported to date are quinonoids, such as NSC663284[7] and BN82685[21] (Fig. 1), which have IC_{50} values in the high nanomolar range. However, the redox properties of the quinones can generate toxic oxygen species, which may be toxic to normal tissues and thus limit their prospect for application.[20] In addition, many Cdc25 phosphatase inhibitors showed low selectivity against other phosphatases. A novel structural scaffold and selective inhibitor activity are needed for antitumor application of these inhibitors.

Herein, we describe the discovery and structural optimization of N-substituted maleimide fused-pyrazole analogues as a novel series of Cdc25B inhibitors with submicromolar inhibitory potency in vitro against Cdc25B. We also describe the discovery of more simplified 3,5-diacylpyrazol analogues as more potent Cdc25B inhibitors.

The original hit compound **1** was obtained by high-throughput screening of an in-house compound library against a recombinant Cdc25B expressed and purified in our laboratory. Compound **1**, which contains a maleimide fused-pyrazole core, was identified with moderate inhibitory activity of Cdc25B (IC_{50} = 32 μM, Fig. 2).

To understand the structure–activity relationship and improve the potency, we initially prepared a series of compounds with scaffold I, as outlined in Figure 2, in which we changed the substituted group R^1 on the phenyl ring, R^2 on the carbonyl group, and R^3 on the nitrogen of the pyrazole ring.

The general synthetic strategy[23–26] of N-substituted maleimide fused-pyrazole analogues is illustrated in Scheme 1. Starting from

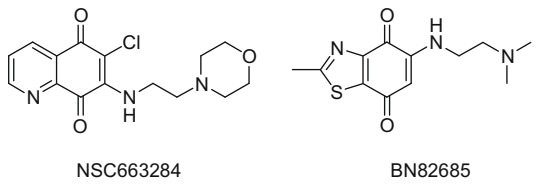

Figure 1. Known Cdc25B inhibitors.

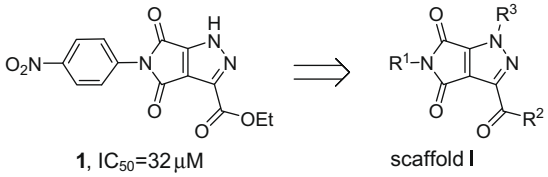

Figure 2. The structure of the original hit compound **1** and new designed scaffold I.

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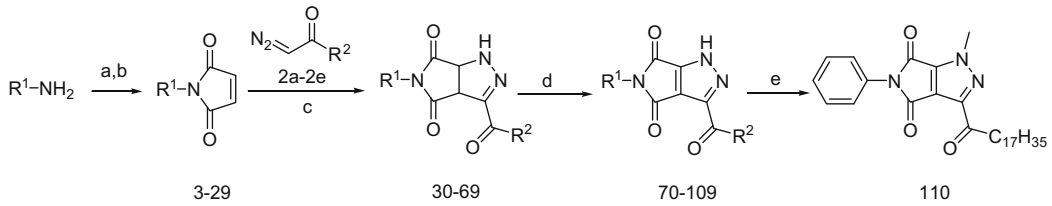


Scheme 1. Reagents and conditions: (a) maleic anhydride, Et_2O, rt; (b) NaOAc, Ac_2O, 120 °C; (c) toluene, 110 °C; (d) MnO_2, acetone, rt; (e) Cs_2CO_3, CH_3I, acetone, rt.

primary amines, acylation with maleic anhydride yielded N-substituted maleimides **3–29**, which were used as dipolarophile to carry out 1,3-dipolar cycloaddition reactions with α-diazo carbonyl compounds **2a–e**[23,27] under refluxing conditions in toluene, and substituted dicyclo-pyrazolines compounds **30–69** were obtained. Treatment of pyrazolines with MnO_2 in acetone provided the desired products **70–109** bearing structural variations in the R^1 and R^2 positions.

For the initial 12 compounds prepared (compounds **70–81**), which have a different R^1 group on the phenyl ring. As shown in Table 1, these modifications led to the total loss of potency except that the 4-Cl-phenyl-substituent compound (compound **76**) displayed nearly the potency of a hit. Therefore, the 4-NO_2-phenyl substituent of compound **1** was retained. Compounds **82–85** were synthesized by replacing R^2. Interestingly, introducing a saturated long carbon chain (compounds **83–85**) improved the potency dramatically, and the 17-carbon chain substitution produced an 80-fold increase in potency (compound **85**, IC_{50} = 0.40 μM).

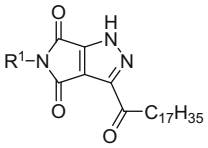
Encouraged by this result, we retained the saturated 17-carbon chain on the right side of the molecule and diversified the R^1 group to synthesize compounds **86–109** using the same synthetic method as described in Scheme 1. The results of the biological activity assay are presented in Table 2 and are summarized as follows. (1) The compound that has no substituent on the phenyl ring (compound **97**, IC_{50} = 0.23 μM) was most potent (compounds **90**, **91**, and **92** showed similar potency). (2) Replacing the phenyl ring with a benzyl or cyclohexyl substituent visibly decreased the potency (compounds **105**, **106**, and **108**). However, compounds **107** and **109**, bearing substituted thiophene and naphthalene at R^1, maintained the inhibitory activity against Cdc25B.

The greatly reduced potency of compound **110** (Table 3), which was synthesized by methylating compound **97** with iodomethane in the presence of cesium carbonate (Scheme 1), suggests that the N–H on the pyrazole ring is essential for the inhibitory activity against Cdc25B.

Table 1
Inhibitory activity for Cdc25B of compounds **70–85**

Compd	R^1	R^2	Cdc25B IC_{50} ± SD (μM)
70	3-NO_2-Ph	OEt	NA
71	2-F-Ph	OEt	NA
72	3-F-Ph	OEt	NA
73	4-F-Ph	OEt	NA
74	2-Cl-Ph	OEt	NA
75	3-Cl-Ph	OEt	NA
76	4-Cl-Ph	OEt	37.4 ± 0.87
77	2-Br-Ph	OEt	NA
78	3-Br-Ph	OEt	NA
79	2-MeO-Ph	OEt	NA
80	4-MeO-Ph	OEt	NA
81	2-MeOOC-Ph	OEt	NA
82	4-NO_2-Ph	Ph	NA
83	4-NO_2-Ph	C_9H_{19}	1.39 ± 0.31
84	4-NO_2-Ph	$C_{13}H_{27}$	0.50 ± 0.12
85	4-NO_2-Ph	$C_{17}H_{35}$	0.40 ± 0.03

Table 2
Inhibitory activity for Cdc25B of compounds **86–109**



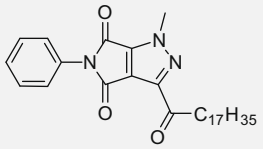
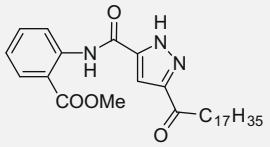
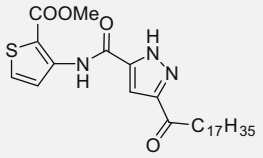
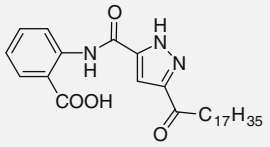
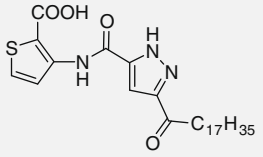
86-109

Compds	R^1	Cdc25B IC_{50} ± SD (μM)
86	3-NO_2-Ph	1.11 ± 0.20
87	2-F-Ph	0.45 ± 0.04
88	3-F-Ph	1.47 ± 0.29
89	4-F-Ph	0.54 ± 0.20
90	3-Cl-Ph	0.24 ± 0.04
91	4-Cl-Ph	0.17 ± 0.01
92	2-Br-Ph	0.25 ± 0.07
93	3-Br-Ph	1.01 ± 0.02
94	4-Br-Ph	0.96 ± 0.03
95	3-MeO-Ph	0.52 ± 0.12
96	4-MeO-Ph	0.54 ± 0.22
97	Ph	0.23 ± 0.01
98	2-Me-Ph	2.13 ± 0.37
99	3-Me-Ph	2.60 ± 0.95
100	4-Me-Ph	4.33 ± 1.09
101	2-MeOOC-Ph	0.41 ± 0.16
102	4-MeOOC-Ph	0.49 ± 0.20
103	2-CF_3-Ph	0.32 ± 0.10
104	3-Cl, 4-F-Ph	1.15 ± 0.25
105	Bn	0.73 ± 0.32
106	4-F-Bn	2.08 ± 0.44
107	2-MeOOC-3-thiophene	0.34 ± 0.08
108	Cyclohexyl	2.92 ± 1.50
109	1-Naphthalene	0.31 ± 0.06

Hydrolysis of compounds **101** and **107** was next tried to introduce carboxylic group on the aromatic ring, but these failed, probably because of the poor stability of the maleimide ring under base conditions. To investigate the relationship between the maleimide ring and Cdc25B inhibitory activity, the maleimide ring was opened and substituted pyrazole compounds **115** and **116** were synthesized using a similar method as described above (Scheme 2). As shown in Table 3, the potency of these two compounds decreased markedly.

Interestingly, the corresponding acid **117**[28] and **118**[29] of compounds **115** and **116** showed more potent inhibitory activity (**117**, IC_{50} = 0.16 μM, 200-fold increase and **118**, IC_{50} = 0.12 μM, 270-fold increase), indicating that introducing a carboxylic group in the left side of the molecule improves the water solubility of the two compounds and increases the potency against Cdc25B. The most important compounds **117** and **118** present a novel and simplified scaffold with more potent inhibitory activity than the original maleimide fused-pyrazole scaffold. Further enzyme kinetic study shown that compound **118** binds reversely with Cdc25B (Fig. 3) and inhibits in a mix-type mode (Fig. 4). These results should provide new ways to modify the structure further and lead to new discoveries.

Table 3
Inhibitory activity for Cdc25B for compounds **110**, **115**–**118**

Compd	Structure	Cdc25B IC_{50} ± SD (μM)
110		30.0 ± 4.41
115		5.08 ± 0.38
116		3.36 ± 0.62
117		0.16 ± 0.01
118		0.12 ± 0.02

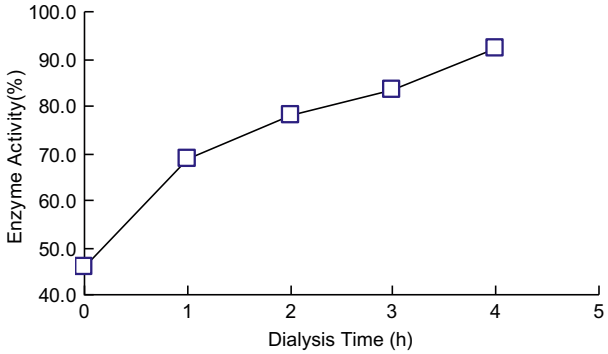


Figure 3. Reversible binding of compound **118** with Cdc25B.[30]

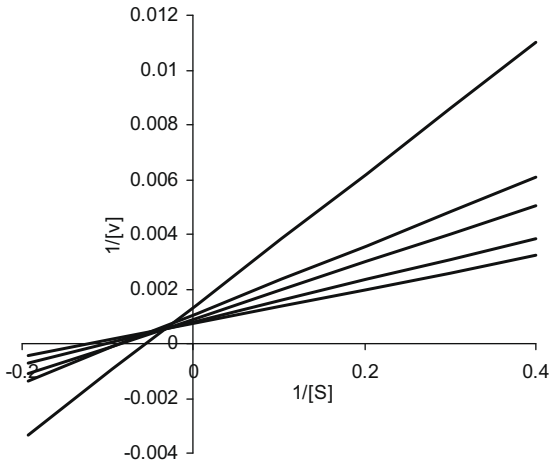
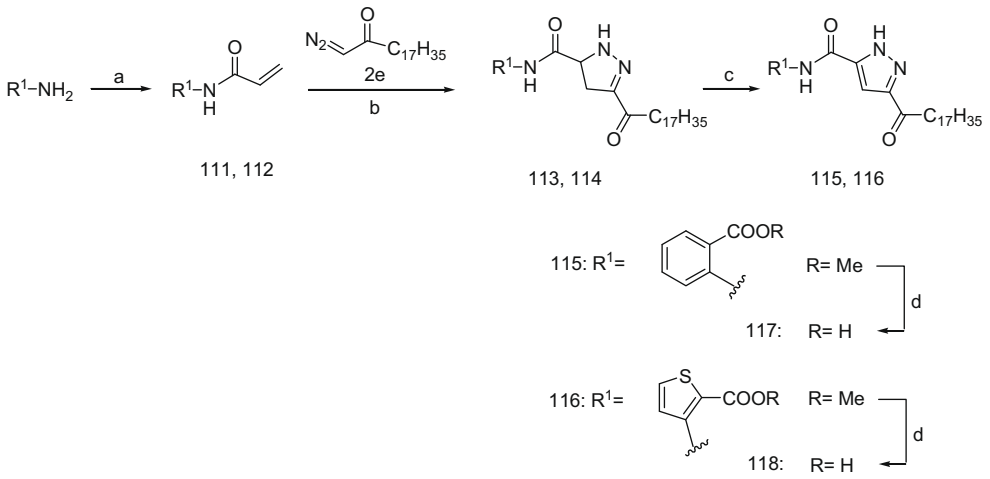


Figure 4. Characterization of compound **118** (0, 0.1, 0.2, 0.3, and 0.4 μM) inhibition.

To study the selectivity of these novel Cdc25B inhibitors for other phosphatases, we also tested the inhibitory activity of six compounds against PTP1B, TCPTP, SHP1, SHP2, and PTP-LAR. The results (Table 4) indicated higher selectivity for Cdc25B than for SHP1, SHP2, and PTP-LAR; minor selectivity for TCPTP; and almost no selectivity for PTP1B. The exception was N-substituted maleimide fused-pyrazole compounds **1** and **97**, which showed moderate selectivity for Cdc25B compared with PTP1B.

In conclusion, novel pyrazole derivatives including N-substituted maleimide fused-pyrazole and 3,5-diacylpyrazole derivatives were identified as Cdc25B inhibitors. Preliminary structural optimization of the series provided compounds **117** (IC_{50} = 0.16 μM) and **118** (IC_{50} = 0.12 μM), which show 200-fold and 270-fold potency of the original hit compound **1** (IC_{50} = 32 μM), respectively, and improved physical properties. These compounds provide a new starting point for further structural modification, which may lead to new discoveries of inhibitory agents against the Cdc25 phosphatases.



Scheme 2. Reagents and conditions: (a) acryloyl chloride, Et_3N, DCM; (b) benzene, 80 °C; (c) MnO_2, acetone, rt; (d) LiOH, THF/H_2O, 0 °C to rt.

Table 4
Inhibitory activity for other phosphatases compared with Cdc25B[31]

Compd	PTP1B IC_{50} ± SD (μM)	TCPTP IC_{50} ± SD (μM)	SHP1 IC_{50} ± SD (μM)	SHP2 IC_{50} ± SD (μM)	PTP-LAR IC_{50} ± SD (μM)	Cdc25B IC_{50} ± SD (μM)
1	NA	NA	NA	NA	NA	31.9 ± 3.08
97	1.56 ± 0.16	3.31 ± 0.19	NA	9.59 ± 0.75	NA	0.23 ± 0.01
115	7.49 ± 1.79	18.7 ± 2.63	NA	NA	NA	5.08 ± 0.38
116	7.16 ± 0.24	18.6 ± 3.17	NA	NA	NA	3.36 ± 0.62
117	0.35 ± 0.03	1.30 ± 0.16	10.6 ± 1.24	15.2 ± 1.97	NA	0.16 ± 0.01
118	0.41 ± 0.02	1.16 ± 0.10	9.79 ± 0.89	12.2 ± 0.76	NA	0.12 ± 0.02

Acknowledgments

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27. Two methods were used to synthesize α-diazo carbonyl compounds **2a**–**e**. Glycine ethyl ester was diazotized by $NaNO_2$ under acidic condition to give ethyl diazoacetate **2a**. Through converting corresponding carboxylic acids to acyl chlorides, then acylating diazomethane, compounds **2b**–**e** were prepared Mulzer, J. In *Comprehensive Organic Functional Group Transformations*; Katritzky, A. R., Meth-Cohn, O., Rees, C. W., Eds.; Pergamon Press: Oxford, 1995. pp 144 and 276.
28. *Characterization data of compound* **117**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 14.40 (s, 1H), 12.40 (s, 1H), 8.76 (d, *J* = 8.1 Hz, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 7.64 (t, *J* = 8.1 Hz, 1H), 7.49 (s, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 2.94 (t, *J* = 6.9 Hz, 2H), 1.52–1.70 (m, 2H), 1.00–1.40 (m, 28H), 0.83 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 191.1, 169.1, 159.5, 147.2, 142.4, 140.4, 133.9, 131.2, 122.7, 119.8, 116.4, 109.2, 39.8, 31.1, 28.9, 28.7 (8C), 28.6, 28.5, 28.4, 23.5, 21.9, 13.7; MS (ESI) *m*/*z* = 498.3 M^++1, 520.3 M^++23, calcd for $C_{29}H_{43}N_3O_4$ (M^+) 497.33.
29. *Characterization data of compound* **118**: ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.5 (s, 1H), 8.13 (d, *J* = 5.1 Hz, 1H), 7.89 (d, *J* = 5.4 Hz, 1H), 7.52 (s, 1H), 2.94 (t, *J* = 6.6 Hz, 2H), 1.50–1.66 (m, 2H), 1.03–1.40 (m, 28H), 0.84 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 191.2, 164.8, 158.1, 146.3, 142.7, 132.3, 121.7, 112.0109.5, 99.4, 39.8, 31.2, 29.0, 28.8, 28.7 (9C), 28.4, 23.5, 22.0, 13.9; MS (ESI) *m*/*z* = 504.1 M^++1, 526.2 M^++23, calcd for $C_{27}H_{41}N_3O_4S$ (M^+) 503.28.
30. The reversible binding assay were carried out by preincubation 0.12 μM compound **118** with Cdc25, then dialyzed for indicated time for activity recovery.
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