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Synthesis of small molecule CDC25 phosphatases inhibitors

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Abstract—A targeted library of small molecules has been prepared to optimize the biological activity of BN82002, our initial lead compound, recently described as an original inhibitor of CDC25 phosphatases. Some of these compounds inhibit CDC25 in the micromolar range and therefore reinforce the interest of CDC25 as an anticancer target.

1. Introduction

Protein phosphorylation/dephosphorylation is one of the most important mechanisms used by cells to regulate crucial biological events such as metabolism, gene expression, cell division, differentiation and development. CDC25 phosphatases, dual specificity enzymes, which can dephosphorylate both phospho-Ser/Thr and phospho-Tyr residues,[1] are essential regulators by dephosphorylating and activating CDK/cyclin complexes at key transition steps of the cell cycle. It has been shown that overexpression of CDC25 is characteristic of a number of human cancers[2] and consequently, inhibition of these phosphatases could represent a new and valuable approach in cancer therapy.[3] Although several inhibitors have been described in the literature,[4,5] only a few molecules have shown in vivo efficacy in xenograft models.

To identify new inhibitors, we have established a high-throughput screening of our molecular library, using recombinant human CDC25C[6] and one of the first CDC25 inhibitors described in the literature, Menadione (**1**, Fig. 1), as a positive control.[7]

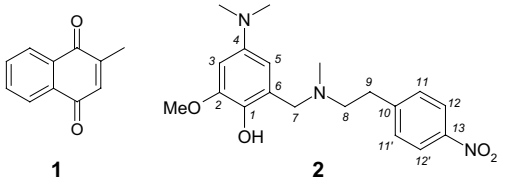

Figure 1. Structure of menadione (**1**) and BN82002 (**2**).

BN82002 (**2**, Fig. 1), was identified as a hit with inhibitory properties at micromolar concentrations, and a family of related compounds has been prepared to explore the structure–activity relationships in the series.

2. Chemistry

Synthesis of BN82002 was performed by reductive amination of 5-(dimethylamino)-2-hydroxy-3-methoxybenzaldehyde (**3**)[8] with *N*-methyl-2-(4-nitrophenyl)-ethylamine (**4**) (Scheme 1).[9,10]

These conditions are well suited for parallel synthesis strategies and in particular, for the use of polymer supported reagents and scavengers. This methodology, starting from a carboxaldehyde (**5**), and an amine (**6**), allows the rapid preparation of secondary or tertiary amines (**7**) containing the required benzylamine moiety (Scheme 2). Aldehydes **5** in the presence of amines **6** lead to an imine intermediate, which is reduced[11] in situ by treatment with borohydride supported on resin.[12]

Keywords: CDC25 inhibitor; Dual specificity phosphatases inhibitor; Antiproliferative agent.
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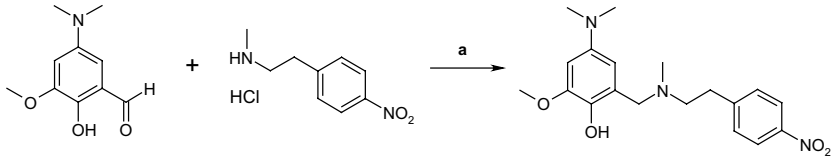
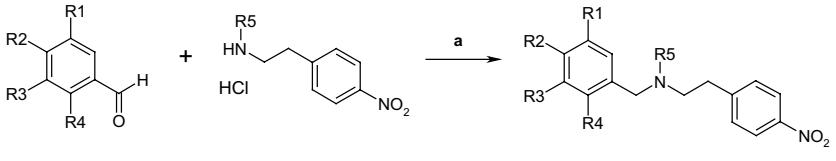


3 4 2

Scheme 1. Synthesis of **2**. Reagents and conditions: (a) (i) **4** (1.1 equiv), Et_3N (1.5 equiv), MeOH, 25 °C, 18 h; (ii) $NaBH_4$ (1.1 equiv), 25 °C, 4 h (34% for steps (i) and (ii)).



5 6 7

Scheme 2. Synthesis of **7**. Reagents and conditions: (a) (i) **6** (1.2 equiv), Et_3N (1.3 equiv), MeOH, 25 °C, 18 h; (ii) polymer supported borohydride (2 equiv), 25 °C, 2 h.

Compounds **7** are obtained directly by this 'one pot' process with acceptable purity using electrophilic scavenger resins: 4-benzyloxybenzaldehyde polystyrene resin,[13] selective for primary amines in the presence of secondary ones and methylisothiocyanate polystyrene resin[14] for secondary amines.[15] Purities were determined by LC/MS.[16] UV purity of the compounds presented is greater than 80%.

3. Results and discussion

Enzyme inhibition was assayed using recombinant fused MBP–CDC25 with 3-*O*-methylfluorescein phosphate (OMFP) as the substrate.[6] In this test, BN82002 (**2**) showed in vitro inhibition of CDC25C phosphatase in a dose dependent manner with an IC_{50} value of 5.4 μM; menadione, under the same conditions, showed an IC_{50} of 18.8 μM.

Using the parallel synthesis methodology described, a series of 2-(4-nitrophenyl)ethylbenzylamines (**7a**–**n**) incorporating the R groups present in BN82002 has been prepared. The starting benzaldehydes were chosen with one, two or three substituents present on the benzyl moiety of the BN82002: dimethylamino, methoxy and hydroxy groups. No activity was observed with the unsubstituted phenyl ring or with monosubstituted compounds (**7a**–**e**, Table 1). Among the disubstituted compounds, hydroxy and methoxy groups at R3 and R4 positions led to a loss of biological activity (**7f**) as did 1,2- and 1,4-dimethoxy and 1-methoxy-3-dimethylamino substitution (**7g**,**h**,**i**). On the other hand, the 1-hydroxy-4-dimethylaminophenyl ring (**7j**) gave appreciable activity. The replacement of the *N*-dimethylamino by a nitro group (**7k**) induced a loss of enzyme inhibition. The trisubstituted phenyl ring: 2-methoxy-3-hydroxy-4-dimethylaminophenyl (**7l**) was moderately active. In summary, positive results were obtained only when the dimethylamino and hydroxy substituents are *para* or *ortho* to each other on the phenyl ring.

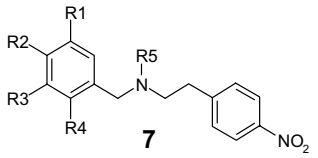
Furthermore, the comparison between **7m** and **7j** and between **7n** and BN82002 (**2**) shows that the secondary amines (**7m**,**n**) are somewhat better CDC25C inhibitors than the corresponding tertiary amines.

BN82002 has been further studied, to confirm the mechanism of action.[17] BN82002 has been shown to inhibit the phosphatase activity of recombinant CDC25A, CDC25B2, CDC25B3, as well as CDC25C through irreversible linkage to its catalytic domain. From a mechanistic point of view, it has been checked by western-blot analysis that BN82002 inhibits tyrosine dephosphorylation on CDK1, the natural CDC25C substrate, and thereby inactivates the CDK1/cyclin B1 complex in cultured Mia PaCa-2 cells. In addition, it has been shown, in synchronized HeLa cells, that BN82002 delays cell cycle progression at G1/S, in S-phase and at the G2/M transition. This observation is in accordance with BN82002 being active against several members of the CDC25 phosphatase family. Thus, cell cycle inhibition is observed at the various stages of the cell cycle where CDC25 phosphatases activities are thought to be required.

On the basis of the targeted mechanism of action, inhibitors of CDC25 such as BN82002 and the other inhibitors of the series should prevent cell proliferation of a broad spectrum of cell types. Preliminary results have confirmed that compounds with significant CDC25 inhibitory activity are antiproliferative in cell culture assays.[18] Studies on the profile of the antiproliferative activity are on-going as a prelude to the choice of appropriate xenograft models.

In conclusion, through the synthesis of a series of analogs of our CDC25 inhibitor, BN82002, we have identi-

Table 1. CDC25C inhibition by a series of general structure (**7**) (IC_{50}: 50% inhibitory concentration)



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Compound	R1	R2	R3	R4	R5	IC_{50} (μM)[a]
BN82002 (**2**)	NMe_2	H	OMe	OH	Me	5.4 ± 1.2
7a	H	H	H	H	Me	NA[b]
7b	NMe_2	H	H	H	Me	NA
7c	H	NMe_2	H	H	Me	NA
7d	H	H	OMe	H	Me	NA
7e	H	H	H	OH	Me	NA
7f	H	H	OMe	OH	Me	NA
7g	H	H	OMe	OMe	Me	NA
7h	OMe	H	H	OMe	Me	NA
7i	H	NMe_2	H	OMe	Me	NA
7j	NMe_2	H	H	OH	Me	5.6 ± 1.6
7k	NO_2	H	H	OH	Me	NA
7l	NMe_2	OH	OMe	H	Me	20.7 ± 0.6
7m	NMe_2	H	H	OH	H	3.9 ± 0.4
7n	NMe_2	H	OMe	OH	H	4.0 ± 0.6
Menadione (**1**)						18.8 ± 1.1

[a] The IC_{50} values reported are calculated from at least two independent experiments. Data represent the mean ± SEM.
[b] NA: not active, $IC_{50} > 80\,\mu M$.

fied the key moiety of the molecule, and have prepared, using parallel synthesis strategy, further inhibitors of phosphatase CDC25C. Studies are in progress to evaluate their in vitro and in vivo behaviour. These results reinforce the interest of considering inhibiting cell cycle driving enzymes as a therapeutic strategy in oncology.

Acknowledgements

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10. Compound **2**: **3** (1 g, 5.12 mmol) was added to a mixture of **4** (1.22 g, 5.63 mmol) and triethylamine (1.1 mL, 7.7 mmol) in anhydrous methanol (30 mL) at room temperature under argon, and the resulting mixture was stirred for 18 h. Sodium borohydride (0.213 g, 5.63 mmol) was then added and stirring was maintained for a further 4 h. The reaction mixture was then partitioned between cold water (10 mL) and dichloromethane (50 mL), and the aqueous phase was further extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered and concentrated to give 1.10 g of a brown viscous oil. Purification by chromatography on silica gel using 3% methanol in dichloromethane gave 0.626 g (34%) of **2** as a brown solid, mp 92–93 °C; ^{1}H NMR (DMSO): δ 8.90 (s, 1H, OH), 8.15–8.12 (d, 2H, $J = 8.7$ Hz, 12, 12′-H), 7.52–7.50 (d, 2H, $J = 8.7$ Hz, 11, 11′-H), 6.28 (s, 1H, 3-H), 6.04 (s, 1H, 5-H), 3.70 (s, 3H, OCH_3), 3.57 (s, 2H, 7-CH_2), 2.94 (t, 2H, $J = 7.1$ Hz, 8-CH_2), 2.74–2.70 (m, 8H, 9-CH_2, $N(CH_3)_2$), 2.21 (s, 3H, NCH_3).
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12. Borohydride, polymer supported (on Amberlite® IRA-400), supplier: Aldrich Chemical Company, Inc.
13. 4-Benzyloxybenzaldehyde polystyrene HL (200–400 mesh), 2% DVB, supplier Novabiochem.
14. Methylisothiocyanate polystyrene HL (200–400 mesh), 2% DVB, supplier Novabiochem.
15. Compounds **7a**–**n**: 50 μmol of aldehyde **5** was added to a mixture of amine **6** (60 μmol, 1.2 equiv) and triethylamine (66 μmol, 1.3 equiv), in anhydrous methanol (1 mL) and the resulting mixture was stirred at room temperature for 18 h. Borohydride (100 μmol, 2 equiv) supported on resin were then added and stirring was maintained for 2 additional

hours. Compounds **7a**–**l**: 60 μmol (1.2 equiv) of 4-benzyloxybenzaldehyde polystyrene resin was added and the mixture stirred for 18 h at room temperature. Compounds **7m** and **7n**: 60 μmol (1.2 equiv) of methylisothiocyanate polystyrene resin was added and the mixture stirred for 18 h at room temperature. In both cases, the resins were filtered and washed with MeOH. The solvent was evaporated and compounds **7a**–**n** were obtained as yellow oils.

16. Mass spectra were acquired on a single quadrupole electrospray mass spectrometer (Micromass, Platform model) and HPLC retention times were acquired on an HPLC system (HP 1100) equipped with a photodiode array UV detector.
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