Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 649–652

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Solid Phase Synthesis of Tetrahydroisoquinolines Having cdc25B Inhibitory Activity

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Received 5 November 1999; accepted 28 January 2000

Abstract—A solid phase synthesis of substituted tetrahydroisoquinolines was developed and used to prepare directed libraries of compounds for screening against the protein phosphatase, CDC25B. From these libraries, a compound was found having approximately a 4-fold improvement in activity.

Combinatorial chemistry has been shown to be an effective tool that aids in both the discovery of new drug leads as well as in the optimization of lead compounds into potential therapeutic agents.[1–3] Augmenting the ease with which chemists can synthesize libraries containing hundreds or even thousands of compounds, has been the explosive growth in the solid phase synthesis of small organic molecules, which brings ease of work-up, purification and automation to organic synthesis.[3–6] Herein we describe the utilization of parallel synthesis on solid support for lead optimization of a template having inhibitory activity against the protein phosphatase, cdc25B. This protein is a member of a specific family of protein phosphatases that catalyze the dephosphorylation and activation of cyclin dependent kinases, a step necessary for cells to progress into mitosis.[7] Inhibition of cdc25B could prove therapeutically useful as a treatment for cancer.[8]

Broad screening of the research compound collection at Pharmacia & Upjohn identified a number of compounds having inhibitory properties against cdc25B.[9] One such compound, **1** (**PNU-108937**) (Fig. 1). was shown to have an IC_{50} of 70–100 μM against cdc25B, and was attractive to us as a starting point to develop libraries of compounds using a solid phase synthesis approach for a number of reasons.[10] First of all, the carboxylic acid moiety at C-3 offers a convenient handle for attachment of a suitable precursor to a solid support. By choosing a suitably protected precursor, one should be able to vary in a combinatorial sense, the acyl group at N-2 and the functionality attached to the C-7 phenol. Finally, we had hoped to draw on the extensive literature for the preparation of peptides on solid support, since **1** can be derived from a constrained tyrosine scaffold.

With these considerations in mind, tetrahydroisoquinoline **3**, appeared to us to be a suitable precursor. The starting tetrahydroisoquinoline, **3**, was prepared from commercially available 3,5-diiodo-L-tyrosine using a Pictet–Spengler cyclization in yields of 45–51%,[11] followed by protection of the amine with the FMOC group and protection of the phenolic hydroxyl as the *t*-butyldimethylsilyl ether (Fig. 2).

This afforded the starting scaffold **3** in good overall yields. Attachment of **3** to Wang resin was best accomplished using the coupling agent, 2-chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate (CIP).[12] Using this reagent and three equivalents of **3**, reproducible yields of 69% were obtained.[13] However these conditions led to complete racemization of the tetrahydroisoquinoline as determined by chiral HPLC. Since both enantiomers of our lead compound were equipotent inhibitors of cdc25B, the racemization was not a concern to us. Other standard coupling conditions either gave poor yields (38% with DCC/DMAP/HOBT), or required six equivalents of the carboxylic acid to achieve a useful yield (60% with Ph_3P/DEAD).

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0960-894X/00/$ - see front matter
PII: S0960-894X(00)00069-X

With functionalized resin **4**, generation of a small test library was straightforward (Fig. 3). After removal of the FMOC group, the secondary amine can be coupled with carboxylic acids using standard peptide coupling conditions.[14] For each carboxylic acid used, the resin obtained was then divided into four separate reactors for completion of the library synthesis. This entailed deprotection of the phenol with tetrabutylammonium fluoride, followed by alkylation with one of the benzyl chlorides. In this manner, 24 discrete compounds were prepared. Cleavage of the products from the resin was accomplished with 95% TFA to afford the desired compounds which were analyzed by HPLC and MS. In most cases the product obtained gave a single peak by HPLC. The presence of the desired molecular ion was verified by mass spectroscopy.

The results obtained from screening this library for inhibition against cdc25B are shown in Table 1.[15] The data show that a number of these new compounds had activity equal to our lead compound, **PNU-108937**. One compound, Entry 12, showed increased inhibitory activity, with an IC_{50} of 15 μM. This compound (R^1 = 3-trifluoromethylcinnamoyl and R^2 = 2,4-dichlorobenzyl)

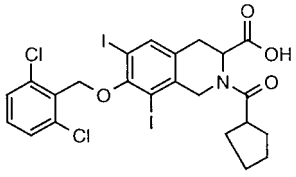

Figure 1. PNU-108937, **1**.

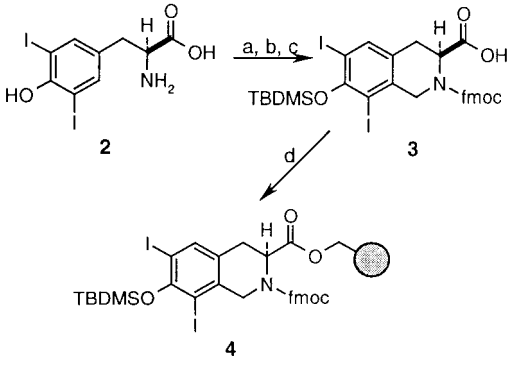

Figure 2. (a) HCHO/HCl/DME 80 °C (40–51%); (b) FMOC-O-Su/Na_2CO_3 (90%); (c) TBDMSCl/Me-Imidazole/THF (91%); (d) Wang Resin (0.82 meq/gm)/CIP/ DMF/pyridine (69%).

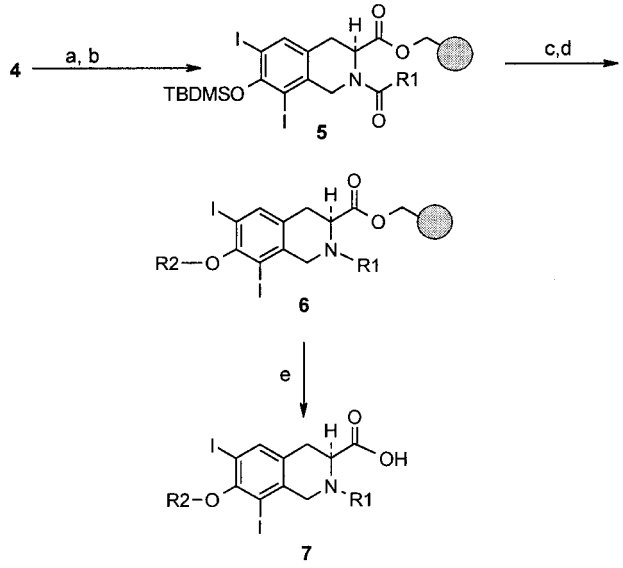

Figure 3. (a) 20% piperidine/DMF; (b) R^1COOH/PyBOP/NMM/DMF; (c) nBu_4NF/DMF; (d) R^2CH_2Cl/iPr_2NEt/DMF, 70 °C; (e) 95% TFA/H_2O.

Table 1. CDC25B inhibition of a library containing 24 compounds of general structure **7** (IC_{50} in μM)

Entry no.	R^1	R^2	Exact mass	Ion found (m-H)$^-$	Area (%)	CDC25B IC_{50} (μM)
1	Hydrocinnamoyl	2,6-Dichlorobenzyl	734.89	733.40	95	>100.00
2	3-Chlorocinnamoyl	2,6-Dichlorobenzyl	766.83	765.00	82	20
3	3,4-Dihlorocinnamoyl	2,6-Dichlorobenzyl	800.79	799.00	88	55
4	2-Chloronicotinoyl	2,6-Dichlorobenzyl	741.81	740.20	43	45
5	3-Fluorocinnamoyl	2,6-Dichlorobenzyl	750.86	749.30	76	45
6	3-Trifluoromethylcinnamoyl	2,6-Dichlorobenzyl	800.86	799.50	88	35
7	Hydrocinnamoyl	2,4-Dichlorobenzyl	734.89	733.50	65	50
8	3-Chlorocinnamoyl	2,4-Dichlorobenzyl	766.83	767.50	54	35
9	3,4-Dichlorocinnamoyl	2,4-Dichlorobenzyl	800.79	799.40	81	30
10	2-Chloronicotinoyl	2,4-Dichlorobenzyl	741.81	740.00	60	>100.00
11	3-Fluorocinnamoyl	2,4-Dichlorobenzyl	750.86	749.40	73	40
12	3-Trifluoromethylcinnamoyl	2,4-Dichlorobenzyl	800.86	799.40	75	15
13	Hydrocinnamoyl	2-Fluorobenzyl	684.95	683.50	87	80
14	3-Chlorocinnamoyl	2-Fluorobenzyl	716.90	715.40	53	60
15	3,4-Dichlorocinnamoyl	2-Fluorobenzyl	750.86	749.30	38	75
16	2-Chloronicotinoyl	2-Fluorobenzyl	691.88	690.40	46	90
17	3-Fluorocinnamoyl	2-Fluorobenzyl	700.93	699.50	55	75
18	3-Trifluoromethylcinnamoyl	2-Fluorobenzyl	750.93	749.40	87	45
19	Hydrocinnamoyl	3-Trifluoromethylbenzyl	734.95	733.40	82	50
20	3-Chlorocinnamoyl	3-Trifluoromethylbenzyl	766.90	765.90	79	40
21	3,4-Dichlorocinnamoyl	3-Trifluoromethylbenzyl	800.86	799.40	82	75
22	2-Chloronicotinoyl	3-Trifluoromethylbenzyl	741.88	740.40	53	30
23	3-Fluorocinnamoyl	3-Trifluoromethylbenzyl	750.93	749.40	78	45
24	3-Trifluoromethylcinnamoyl	3-Trifluoromethylbenzyl	800.92	799.50	83	50

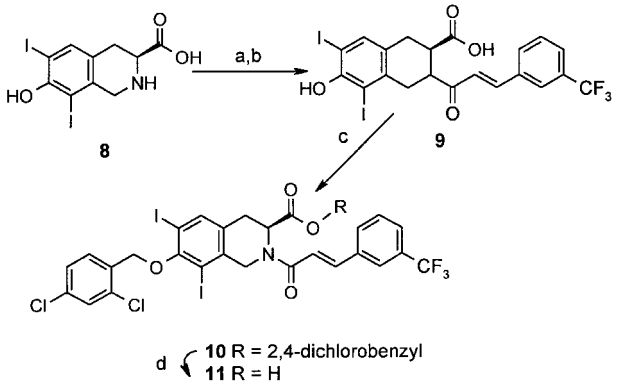

Figure 4. (a) BSTFA/iPr_2NEt/CH_3CN; (b) 3-trifluoromethylcinnamoyl chloride (48%); (c) α,2,4-trichlorotoluene/iPr_2NEt/DMF, 70 °C (63%); (d) 0.25 N NaOH/THF (82%).

was resynthesized according to Figure 4 for complete characterization. Thus, solubilizing **8** in acetonitrile with BSTFA followed by acylation with 3-trifluoromethylcinnamoyl chloride afforded **9**. Alkylation with α,2,4-trichlorotoluene provided the ester **10**, which was saponified to afford the desired compound **11**. An analytically pure sample of **11** had an IC_{50} of 23 μM against cdc25B, a value that was within experimental error of the value obtained for the same compound when tested as a member of a library.

Encouraged by these results, a larger library was prepared, having a variety of cinnamoyl substituents at N-2 and alkyl substituents at the C-7 phenol. The compounds prepared are listed in Table 2. The presence of each targeted molecular ion was verified by mass spectroscopy and, the targeted structure was shown to

Table 2. The inhibition of CDC25B by tetrahydroisoquinolines having general structure **7**

Entry no.	R^1	R^2	Exact mass	Ion found $(m\text{-}H)^-$	Area (%)	IC_{50} in μM (% inhibition at 100 μM)
1	4-Chlorocinnamoyl	2,4-Dichlorobenzyl	766.83	765.80	44	35
2	4-Nitrocinnamoyl	2,4-Dichlorobenzyl	777.85	776.80	41	45
3	2-Fluorocinnamoyl	2,4-Dichlorobenzyl	750.86	749.80	60	(32)
4	2,4-Methylenedioxycinnamoyl	2,4-Dichlorobenzyl	776.86	775.70	48	60
5	4-Methylcinnamoyl	2,4-Dichlorobenzyl	746.89	745.80	54	27
6	4-Chlorocinnamoyl	*m*-Trifluoromethylbenzyl	766.90	765.80	68	(22)
7	4-Nitrocinnamoyl	*m*-Trifluoromethylbenzyl	777.92	776.80	49	50
8	2-Fluorocinnamoyl	*m*-Trifluoromethylbenzyl	750.93	749.80	61	35
9	2-Trifluoromethylcinnamoyl	*m*-Trifluoromethylbenzyl	800.92	799.80	66	36
10	2,4-Methylenedioxycinnamoyl	*m*-Trifluoromethylbenzyl	776.93	775.80	24	66
11	4-Methylcinnamoyl	*m*-Trifluoromethylbenzyl	746.95	745.80	47	30
12	4-Chlorocinnamoyl	*p*-trifluoromethybenzyl	766.90	765.80	64	30
13	3,4,5-Trimethoxycinnamoyl	*p*-trifluoromethybenzyl	822.97	821.80	61	57
14	4-Nitrocinnamoyl	*p*-trifluoromethybenzyl	777.92	776.90	34	55
15	2-Fluorocinnamoyl	*p*-trifluoromethybenzyl	750.93	749.80	47	55
16	3-Fluorocinnamoyl	*p*-trifluoromethybenzyl	750.93	749.80	53	30
17	2-Trifluoromethylcinnamoyl	*p*-trifluoromethybenzyl	800.92	799.80	58	27
18	2,4-Methylenedioxycinnamoyl	*p*-trifluoromethybenzyl	776.93	775.80	61	32
19	4-Methylcinnamoyl	*p*-trifluoromethybenzyl	746.95	745.80	66	35
20	4-Chlorocinnamoyl	3-Methoxybenzyl	728.92	727.80	22	75
21	3,4,5-Trimethoxycinnamoyl	3-Methoxybenzyl	784.99	783.90	26	(25)
22	4-Nitrocinnamoyl	3-Methoxybenzyl	739.94	738.80	16	59
23	2-Fluorocinnamoyl	3-Methoxybenzyl	712.95	711.80	20	(34)
24	3-Fluorocinnamoy	3-Methoxybenzyl	712.95	711.80	23	(41)
25	2-Trifluoromethylcinnamoyl	3-Methoxybenzyl	762.95	761.80	18	(28)
26	2,4-Methylenedioxycinnamoyl	3-Methoxybenzyl	738.95	737.80	28	58
27	4-Methylcinnamoyl	3-Methoxybenzyl	708.97	707.80	38	62
28	Dichlorocinnamoyl	2,4-Dichlorobenzyl	800.79	799.3	83	23
29	4-Bromocinnamoyl	2,4-Dichlorobenzyl	810.78	809.3	77	23
30	alpha-Methylcinnamoyl	2,4-Dichlorobenzyl	746.89	745.5	74	40
31	alpha-Fluorocinnamoyl	2,4-Dichlorobenzyl	750.86	749.4	67	36
32	3-Bromocinnamoyl	2,4-Dichlorobenzyl	810.78	809.3	77	40
33	2-Bromocinnamoyl	2,4-Dichlorobenzyl	810.78	809.3	83	33
34	2-Chlorocinnamoyl	2,4-Dichlorobenzyl	766.83	765.4	86	15
35	2,4-Dichlorocinnamoyl	Phenylmethyloxycarbonylmethyl	790.88	789.4	44	28
36	4-Bromocinnamoyl	Phenylmethyloxycarbonylmethyl	800.86	799.4	63	90
37	alpha-Methylcinnamoyl	Phenylmethyloxycarbonylmethyl	736.97	735.5	53	(24)
38	alpha-Fluorocinnamoyl	Phenylmethyloxycarbonylmethyl	740.94	739.5	51	90
39	3-Bromocinnamoyl	Phenylmethyloxycarbonylmethyl	800.86	799.4	67	90
40	2-Bromocinnamoyl	Phenylmethyloxycarbonylmethyl	800.86	799.4	20	45
41	alpha-Cyanocinnamoyl	Phenylmethyloxycarbonylmethyl	747.95	894	38	(36)
42	2-Chlorocinnamoyl	Phenylmethyloxycarbonylmethyl	756.91	755	30	70
43	2,4-Dichlorocinnamoyl	3-Chloropropyl	718.83	718.8	88	52
44	4-Bromocinnamoyl	3-Chloropropyl	728.82	728.8	82	70
45	alpha-Methylcinnamoyl	3-Chloropropyl	664.92	664.9	62	(12)
46	alpha-Fluorocinnamoyl	3-Chloropropyl	668.90	668.9	76	100
47	3-Bromocinnamoyl	3-Chloropropyl	728.82	728.8	78	70
48	2-Bromocinnamoyl	3-Chloropropyl	728.82	728.8	79	33
49	alpha-Cyanocinnamoyl	3-Chloropropyl	675.90	675.9	40	(11)
50	2-Chlorocinnamoyl	3-Chloropropyl	684.87	684.9	78	27
51	alpha-Cyanocinnamoyl	7-Hydroxy	599.90	598.60	44	(12)

be present as the major component of the product mixture. The majority of the compounds prepared showed inhibitory activity either equal to or better than our lead compound, **PNU-108937**.

In conclusion, we have developed a solid phase synthesis of substituted tetrahydroisoquinolines that have shown activity as inhibitors of the protein phosphatase cdc25B. The starting scaffold is readily obtained from the commercially available 3,5-diiodotyrosine in three steps, and allows for the incorporation of two elements of diversity via acylation of the N-2 position and alkylation of the C-7 phenol. A small test library was prepared and a compound was discovered having increased inhibition against cdc25B, thus illustrating the usefulness of this route for the rapid preparation of new analogues having increased activity. Larger libraries can be readily prepared incorporating a number of diverse, commercially available carboxylic acids and alkylating agents. Using alternate linker strategies, one could easily introduce a third element of diversity at the C-3 position.

Acknowledgements

The authors would like to acknowledge Martin A. Deibel, Cindy L. Wolfe and Anthony W. Yem for the preparation of the cdc25B enzyme construct used in the inhibition assays.

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15. Crude products were submitted for assay and the weights are uncorrected to account for purity. Compounds dissolved in DMSO were diluted with assay buffer (50 mM Tris–HCl pH 8.0, 50 mM NaCl, 1 mM EDTA, 10 mM DTT, 0.005% Tween 20) containing the substrate *p*-nitrophenyl phosphate (pNPP; Sigma Chemical Company, St. Louis, MO) in wells of 96-well Immulon 2 microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). Assay buffer containing $cdc25B_{356–556}$ (obtained from Anthony Yem, Cindy Wolfe and Marty Deibel) was added to each well, the plate was placed on an orbital shaker for 10 s, and then the resultant optical densities in each well were monitored at 405 nm for 30 min at 1 min intervals at 25 °C using a SPECTRAmax™ 250 microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA). Final assay concentrations were 0 to 100 μM compound, 5 mM pNPP, 100 nM $cdc25B_{356–556}$, and 5% DMSO in a volume of 200 μL.