Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2581–2584

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Solid-Phase Synthesis of a Library Constructed of Aromatic Phosphate, Long Alkyl Chains and Tryptophane Components, and Identification of Potent Dipeptide Telomerase Inhibitors

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Received 4 June 2001; accepted 14 July 2001

Abstract—Telomerase inhibitors are expected as a new candidate of therapeutic agents for cancer. Recently, we have found novel inhibitors based on the bisindole skeleton. In this study, solid-phase synthesis was applied to construct a library of inhibitors having aromatic phosphate, long alkyl chain and tryptophane components, from which a D,D-ditryptophane derivative has been identified as a new potent telomerase inhibitor with IC_{50} values of 0.3 μM. A hypothetical binding model for the new inhibitors has been proposed based on the structure–activity relationship.

Telomerase is the enzyme to maintain telomere length, and its activity is not observed in normal somatic cells.[1] In contrast, high expression of telomerase is observed in around 85–90% of human tumor cells; therefore, telomerase is regarded as a specific target for development of cancer chemotherapeutic agents.[2] There are several types of inhibitors. Antisense oligodeoxynucleotides and related compounds exhibit potent inhibition of telomerase in the picomolar range.[3] Small molecules that bind the G-quartet structure of telomere also show inhibitory activity.[4] Some new natural products or derivatives of known compounds have been identified as potent inhibitors.[5] In spite of intensive research, there have been no clinical trials of inhibitors to date, and discovery of novel inhibitors will contribute to evaluation of telomerase inhibitors for cancer chemotherapy. Recently, we have developed new telomerase inhibitors (**1**) based on the bisindole unit (Fig. 1).[6] The new inhibitors are constructed of a simple assembly of some structural units: (i) a phosphate with a hydrophobic group; (ii) a bisindole unit; and (iii) a long alkyl spacer between them. Such a simple structural feature of the inhibitors led us to search for more potent inhibitors based on a solid-phase synthesis that may be applied to construct an inhibitor library. Here, we wish to report that a potent inhibitor has been identified from the compounds obtained by solid-phase synthesis. Also, we wish to propose hypothetical binding sites for this class of inhibitors based on the structure–activity relationship.

In order to construct a library of compounds that have a phosphate group at the terminal, we chose a sulfone linker to the Merrifield resin.[7] Use of a variety of components for sequential introduction of an alkyl chain and indole units would produce a compound library (Fig. 2). Linkage of different units would be done by amide formation. Thus, we used aromatic amino acids such as phenylalanine or tryptophane as a replacement of the bisindole unit. An example of solid-phase synthesis of inhibitors is shown in Figure 3. At first, mercaptoethanol was introduced to the Merrifield resin, followed by oxidation with *m*CPBA to form the sulfone linker (**3**). The peaks at 3450, 1289 and 1111 cm^{-1} in the

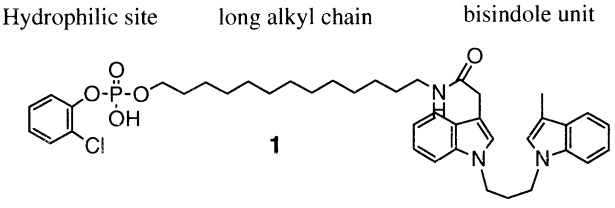

Figure 1. A lead structure of telomerase inhibitor with bisindole unit.

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PII: S0960-894X(01)00507-8

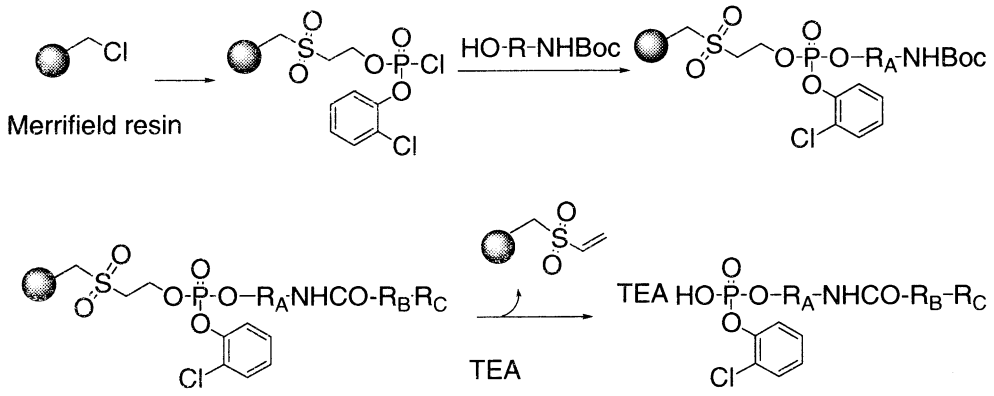


Figure 2. Synthetic plan of solid-phase synthesis of inhibitor library.

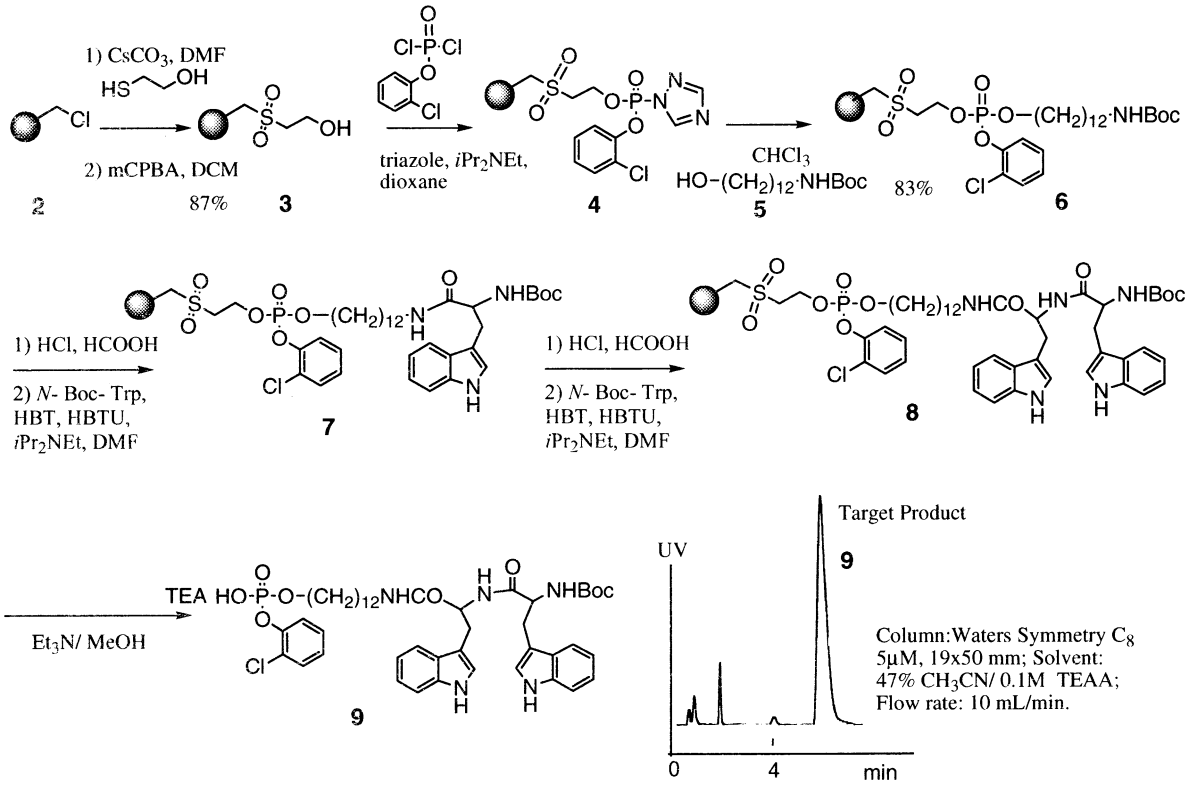
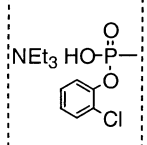
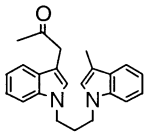
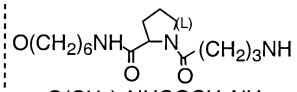
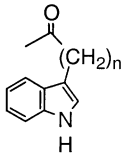
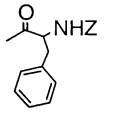
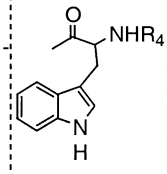
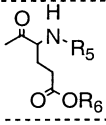
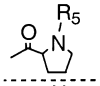
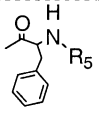
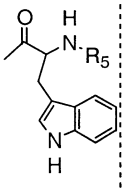


Figure 3. An example of solid-phase synthesis of telomerase inhibitors.

FT-IR spectrum of **3** indicated the formation of sulfonylethanol group. Introduction of a phosphate group was accomplished using *o*-chlorophenylphosphoroditriazoate prepared from *o*-chlorophenylphosphorodichloride by treatment of triazole and triethylamine. Triazoate-containing resins (**4**) were reacted with *N*-Boc aminoalkylalcohol (**5**) to form phosphodiester linkage (**6**) in good yield. An activator such as *N*-methylimidazole was not used, because it disturbed this reaction by promoting β-elimination of the sulfone linker. The *N*-Boc group of **6** was deprotected by HCl in HCOOH, followed by amide bond formation with *N*-Boc tryptophane with HBTU-HBT as the coupling agent to enable the introduction of the first indole unit (**7**). The second tryptophane unit was introduced in the same manner to give **8**, and finally, the desired compound **9** was cleaved from the resin by treatment with TEA. The crude product was directly purified by HPLC to give the desired compound **9** in more than 99% purity in 24% overall yield from the Merrifield resin.

Inhibitory activity of all the synthesized compounds was tested by a quantitative stretch PCR assay[8] with the use of telomerase extracted from HCT116 (American Type Culture Collection) and the results are summarized in Table 1. Structural units are named as R_1–R_6 from the phosphate group, and the alkyl spacer to the aromatic part. It was apparent that the alkyl spacer needs some length, and *n*-dodecyl gave the best activity in this study (**1** vs **10**, or **17**, **18** vs **19**, **20**). The hydrophobic nature of the alkyl spacer is also an important factor from the fact that either the ether spacer (**11**) or amido spacer (**12** or **13**) diminished activity. Interestingly, the bisindole can be replaced by a monoindole unit (**1** vs **14** and **15**), not by a

Table 1. Structure inhibitory activity (IC_{50}, μM) of new telomerase inhibitors[a]

Compd	R_1	R_2	R_3	R_4	R_5	R_6	Stereochem (R_3, R_4)	IC_{50} (μM)
1		$O(CH_2)_{12}NH$						3.6
10		$O(CH_2)_6NH$						29.4
11		$O(C_2H_4O)_3C_2H_4NH$						>100
12								46.9
13		$O(CH_2)_6NHCOCH_2NH$-$CO(CH_2)_3NH$						>100
14		$O(CH_2)_{12}NH$		n=1				33.9
15				n=3				29.7
16							L	>100
17		$O(CH_2)_6NH$		H			L	>100
18		$O(CH_2)_6NH$		Boc			L	>100
19		$O(CH_2)_{12}NH$		H			L	45.2
20				Boc			L	18.3
21				Ac			L	90.7
22				Bz			L	7.3
23				Z			L	3.6
24				*N*-Z-Gly-			L	12.1
25				H			D	15.1
26				Boc			D	20.7
27					Boc	Bzl	L, L	6.4
28					Boc	H	L, L	>100
29					H	H	L, L	>100
30					Boc		L, L	9.5
31					H		L, L	>100
32					Boc		L, L	6.8
33					H		L, L	22.1
34					Boc		L, D	>100
35					H		L, D	>100
36					H		L, L	13.7
37					H		L, D	~[b]
38					H		D, L	10.6
39					H		D, D	11.1
40(9)					Boc		L, L	18.9
41					Ac		L, L	22.9
42					Boc		L, D	>100
43					Boc		D,L	>100
44					Boc		D, D	0.3
45					H		D, D	>100
46		$O(CH_2)_6NHCOCH_2NH$-$CO(CH_2)_3NH$			Boc		D, D	>100
47					H		D, L	>100
48					Boc		D, L	>100
49					H		L, L	>100
50					Boc		L, L	>100
51					H		L, D	>100
52					Boc		L, D	>100

[a]Determined by the stretch PCR assay.[8]
[b]Not yet determined.

simple benzene ring (**16**), and it turned out that tryptophane may be used as a component. In the series of mono L-tryptophane derivatives (**17**–**24**), the *N*-protecting group influenced the inhibitory potency. The benzyloxycarbonyl protecting group (**23**) gave the best activity with $IC_{50} = 3.6\,\mu M$, exhibiting almost the same potency with **1**. Inhibitory potency was not affected by the stereochemistry of tryptophane (**20** vs **26**). An additional amino acid component was introduced to the amino group of the first tryptophane to form dipeptide-type inhibitors (**27**–**35**). An apparent tendency was observed for the second amino acid components, that is, an aromatic and bulky unit induced high inhibitory activity (**27**, **30** or **32**). In the dipeptide-type inhibitors, stereochemistry of the amino acid significantly affected the activity. For example, high activity of the L,L-isomer of **32** and **33** disappeared in the L,D-isomer **34** and **35**. In contrast, stereochemistry had no effect in tryptophane–tryptophane (Trp-Trp) dipeptides without an *N*-protecting group (**36**–**39**). A remarkable effect of the stereochemistry was observed for *N*-Boc-protected Trp-Trp dipeptides (**40**–**44**). The L,D- (**42**) or D,L-isomer (**43**) did not show inhibitory activity, that of the L,L-isomer (**40**) was moderate, and the D,D-isomer (**44**) exhibited the most potent activity with IC_{50} of $0.3\,\mu M$, which is the best value obtained in this study. The dependency on the stereochemistry has suggested that there should be some stereospecific demand in the enzyme binding sites for this series of inhibitors. Inhibitory activity was lost by the replacement of the spacer by the amide spacer (**45**–**52**), again suggesting the importance of the hydrophobic nature of the spacer.

Taking into account the above-mentioned structural requirements for potent inhibitory activity to telomerase, we may propose a hypothetical binding site for the new telomerase inhibitors (Fig. 4). In the previous report, existence of a phosphate group was shown to be essential for inhibitory effect. Also, we have revealed that a component of the terminal phosphodiester needs to be an aromatic or hydrophobic group.[6] The importance of hydrophobic nature of the alkyl spacer has been clearly indicated in this study. In addition to these three pockets, three other binding pockets can be assumed for the indole, aromatic and Boc groups. The latter three pockets may be located in a stereospecific manner in the enzyme binding site. The lead compound **1** can occupy five of the six pockets and exhibits high potency. The components of monotryptophane derivatives may fit to the four binding pockets, and, additionally, the *N*-protecting group may bind to either the Boc or aromatic pocket. For instance, *N*-Z-tryptophane derivative (**23**) might have the five binding components and exhibit high activity. Compared to the D,D-Trp-Trp dipeptide inhibitor that showed the best fit to this model, the D,L- and L,D-isomers should have a quite different conformation, causing much less binding affinity to this model. The L,L-isomer may behave like an enantiomer of D,D-isomer, and binding to the model would be less favorable than the D,D-isomer. As the alkyl chain plays a significant role for inhibitory activity, its conformation should affect inhibitory potency. Further study with the use of molecules that have a fixed conformation will produce more useful information and give rise to more potent inhibitors.

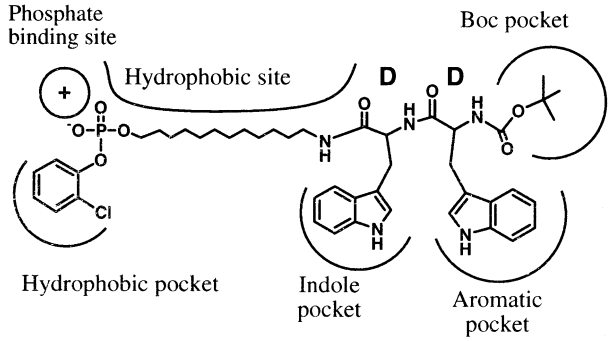


Figure 4. Hypothetical binding pockets for dipeptide-type telomerase inhibitors.

In conclusion, we have synthesized a library of telomerase inhibitors by solid-phase synthesis, and have succeeded in identifing a new potent telomerase inhibitor with a unique structure of the D,D-ditryptophane component. The hypothetical binding model can explain the structure–activity relationship revealed in this study and may to contribute further development of potent telomerase inhibitors.

References and Notes

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