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Development of Novel Telomerase Inhibitors Based on a Bisindole Unit

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Abstract—Telomerase is the enzyme that elongates telomere repeat at the ends of a chromosome. As high telomerase activity is observed in most cancer cells, inhibitors of human telomerase have been expected as new chemotherapeutic agents for cancer. We describe here the discovery of novel inhibitors with IC_{50} values in the submicromolar range. The structure of the novel inhibitors will be useful as a scaffold for construction of the library in the search for telomerase inhibitors.

The DNA sequences at the ends of a chromosome include tandem repeats of the sequence that is specialized by a species such as 5′-TTAGGG in humans. In most human somatic cells, telomeres shorten progressively by 50–200 nucleotides with each cell division.[1] Reduction in the telomere length is believed to lead ultimately to senescence and growth arrest. In contrast, around 85–90% of human tumor cells express telomerase, the enzyme that maintains telomere length, and acquires indefinite replicative capacity leading to cellular immortality. If the telomere lengths of cancer cells were significantly short, selective inhibitors of telomerase would stop proliferation of such cells. In this sense, telomerase is regarded as a specific target for development of cancer chemotherapeutic agents, although stem cells have significant telomerase activity.[2] As there have been no clinical trials of inhibitors to date despite intensive research, discovery of novel inhibitors will contribute to development of new drugs in advanced cancer chemotherapy.

Antisense oligodeoxynucleotides and related compounds exhibit potent inhibition of telomerase in the picomolar range.[3] Development of a delivery method of these oligomers will be needed for clinical applications. Although inhibitors with small molecular weight should be promising candidates in cancer chemotherapy, at least the reported ones are relatively less potent and less selective. As no detailed structure of telomerase has been revealed, several strategies have been attempted for identification of inhibitors of telomerase such as with high-throughput screening, hypothesis on the G-quartet structure of telomere and modification of known inhibitors of DNA and RNA polymerase.[4] Telomerase contains RNA as a template for telomere and is a member of reverse transcriptases; therefore, it seems reasonable to take known inhibitors of transcriptases as lead structures for new inhibitors. Previously, we have identified a novel inhibitor (**1**)[5] of DnaA protein, an initiation factor of DNA replication at *oriC* of *E. coli*, and this compound was found to have inhibitory activity to human telomerase in a preliminary assay. This finding led us to develop novel inhibitors of telomerase. In the present study, we wish to report development of a unique class of telomerase inhibitors.

The 3-acetoxy-2,2′-bi-1*H*-indole derived from indigo was used as the starting material to obtain a variety of derivatives (**1**–**13**). A preliminary study on the SAR (Table 1) has shown the following: (1) the carboxylic acid at the terminus is primarily important (**1** vs **2**, **11** vs **12**); (2) the length of the spacer between the carboxylic acid and the bisindole skeleton should not be shorter or longer than around 14 methylene (**1**, **5**–**9**); (3) 1*N*-alkylation of the indole skeleton is tolerable (**11**, **13**). Although the bisindole skeleton was proven to be a useful lead structure for telomerase inhibitors, it turned out that **1** is labile under acidic condition and easily suffers air oxidation. Then, we used a 3-carboxymethyl indole skeleton (**14**) as an alternative structure of the 3-substituted indole unit. As 1*N*-alkylation was shown to

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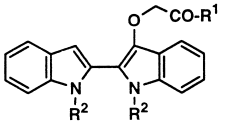
have no effect on inhibitory activity (Table 1, **11** and **13**), we designed new compounds connecting two indole units by 1*N*-alkylation (Scheme 1).

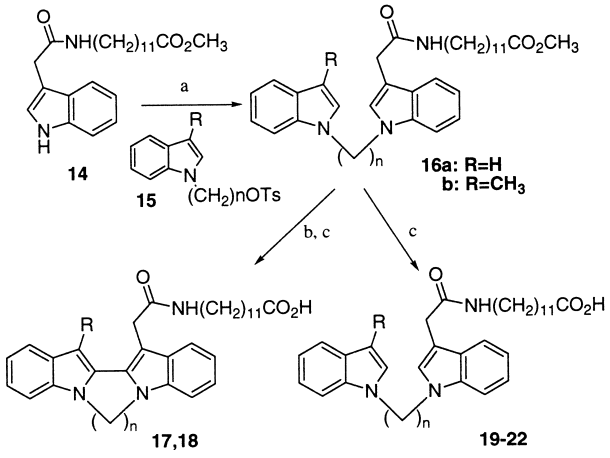
3-Carboxymethylindole was coupled with methyl 12-aminododecanoate to give **14**. Meantime, indole (3-Me or 3-H) was alkylated with TsO-$(CH_2)_2$-OTHP or TsO-$(CH_2)_3$-OTHP, and the product was transformed into the corresponding tosylate (**15**). Alkylation of **14** was then performed with **15** to furnish the linked bisindole derivatives **16**. Intramolecular coupling between the 2,2′-position of **16** was carried out in trifluoroacetic acid (TFA) and the following DDQ oxidation produced the corresponding pentacyclic compounds, which were then hydrolyzed to afford the carboxylic derivative **17** or **18**. The compounds without the 2,2′-linkage of the bisindole (**19**–**22**) were also obtained from **16** by hydrolysis. Mono-indole compounds having a variety of alkyl groups at 1*N* (**24**–**29**) were also synthesized for comparison with bisindole compounds (Scheme 2).

Table 1. Inhibition ratio of telomerase[a]



Compounds			Inhibition (%)
No	R^1	R^2	
1	$NH(CH_2)_{11}COOH$	H	68
2	$NH(CH_2)_{11}COOCH_3$	H	8
3	OH	H	9
4	OCH_3	H	1
5	$NH(CH_2)_2COOH$	H	0
6	$NH(CH_2)_5COOH$	H	0
7	$NH(CH_2)_5CONHCH_2COOH$	H	13
8	$NH(CH_2)_{11}CONHCH_2COOH$	H	36
9	$NH(CH_2)_4CONH(CH_2)_5COOH$	H	56
10	$NH(CH_2)_{11}CONH(CH_2)_{11}COOH$	H	0
11	$NH(CH_2)_{11}COOH$	CH_3	90
12	$NH(CH_2)_{11}COOCH_3$	CH_3	0
13	$NH(CH_2)_{11}COOH$	CH_2Ph	73

[a]Determined by the stretch PCR assay with use of 100 μM of the ligand.

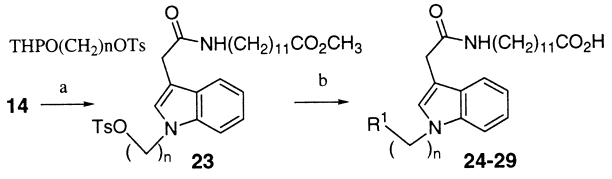


Scheme 1. (a) KO*t*Bu, DMF; (b) (1) TFA, CH_2Cl_2; (2) DDQ, toulene; (c) aqueous NaOH, MeOH, THF.

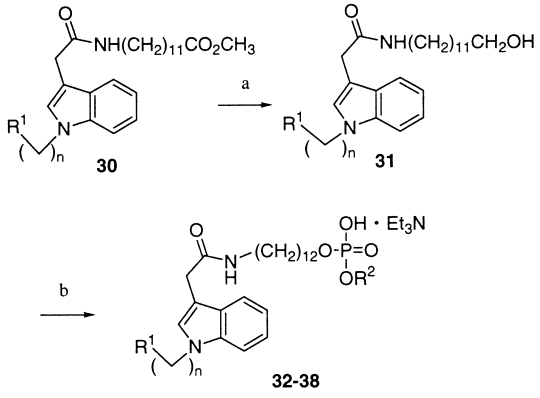
As the terminal carboxyl group should be in its anionic form at physiological pH, it was interesting to us to introduce another anionic group such as a phosphate group. Accordingly, the ester group of **30** was reduced to the corresponding hydroxyl compound (**31**). Phosphodiester derivatives (**32**–**38**) were derived from **31** by the conventional method and purified as its TEA salt (Scheme 3).

Inhibitory activity of all the synthesized compounds was tested by a quantitative stretch PCR assay[6] with the use of telomerase extracted from HCT116 (American Type Culture Collection) and the results are summarized in Tables 2 and 3.

It should be noted that bisindole derivatives having an ethyl or propyl *N*,*N*′-linker (**17** and **18**) exhibited stronger inhibitory activity than the lead compound **1** (Table 2). More surprising was the finding that the compounds (the type **B**; **19**–**22**) without 2,2′-linkage showed almost equal potency with **17** and **18**. The compounds of the type **C** having one indole unit also displayed telomerase inhibition to a similar extent with the type **B** compounds. Substitution of the indole with pyrrole or pyrrolidine (**25** and **26**) diminished inhibitory activity, whereas potency was retained with the compounds having a 1*N*-naphthyloxyethyl (**24**), phenoxypropyl (**28**) or *c*-hexyloxypropyl (**29**) group. These results have suggested that a hydrophobic group of suitable volume may be an essential factor for inhibition potency but not the nitrogen atom. It has been also clearly shown that a 3-carboxymethylindole skeleton (**14**) can be a useful unit for telomerase inhibitors.

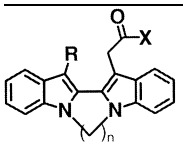
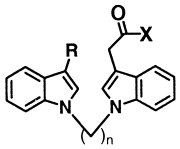
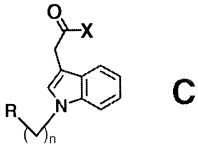


Scheme 2. (a) (1) KO*t*Bu, DMF; (2) TsOH·H_2O, MeOH; (3) TsCl, Et_3N, CH_2Cl_2; (b) (1) nucleophile, KO*t*Bu, DMF; (2) aqueous NaOH, THF.



Scheme 3. (a) $LiAlH_4$, THF; (b) (1) *i*$Pr_2NP(Cl)OCH_2CH_2CN$, *i*Pr_2-NEt, CH_2Cl_2; (2) R^2OH, 1*H*-tetrazole; (3) *t*BuOOH; (4) Et_3N, $CHCl_3$.

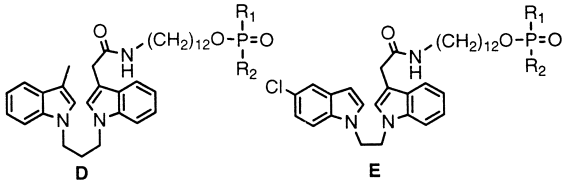
Table 2. Inhibitory activity of indole derivatives[a]

	Compound				Inhibition ratio (%)[b]	IC_{50} (μM)
	No.	Type	R	n		
 A	**1**	—	—	—	68	>100
	17	A	H	2	62	62
	18	A	CH_3	3	82	50
 B	**19**	B	H	2	77	41
	20	B	CH_3	2	7	>100
	21	B	H	3	46	92
	22	B	CH_3	3	100	20
 C	**24**	C	1-Naphthyloxy	2	65	35
	25	C	*N*-Pyrrolyl	3	0	>100
	26	C	*N*-Pyrrolidyl	3	13	>100
	27	C	Phenoxy	2	0	—
	28	C	Phenoxy	3	75	33
	29	C	*c*-Hexyloxy	3	75	67

[a]Determined by the stretch PCR assay.
[b]Inhibition with use of 100 μM of the ligand.

Inhibitory activities of the compounds bearing a phosphodiester group, the type **D** and **E** (**32**–**38**), are summarized in Table 3. Non-anionic compounds (**32** and **33**) did not show significant effect, whereas anionic ones turned out to be potent inhibitors. In particular, **34** and **36** exhibited the strongest inhibition among the compounds tested in this study. The compounds with a relatively large hydrophobic group; *m*-chlorophenoxy of **36**, phenoxy of **37**, *c*-hexyloxy of **38**, are more potent than **35** with a smaller 2-cyanoethyl unit of phosphodiester, indicating that the hydrophobic nature of the phosphodiester terminus is also an important factor for the inhibitory activity. The important role of the 2-chlorophenylphosphodiester group was also shown by the fact that the compound **25** of the type C became a potent inhibitor with $IC_{50} = 4.2$ μM when it was converted to the corresponding 2-chlorophenylphosphodiester structure. The compounds (**34**, **36**–**38**) showed less potency in inhibition of Klenow enzyme with IC_{50} values of 79, 70, 61 and 101 μM, respectively, indicating selective inhibition toward telomerase.

Table 3. Telomerase inhibition by phosphate derivatives[a]


D E

Compounds				Inhibition (%)[b]	IC_{50} (μM)
No	Type	R_1	R_2		
32	D	OCH_2CH_2CN	OPh*m*-Cl	20	>100
33	E	OCH_2CH_2CN	OPh*m*-Cl	6	>100
34	D	OH·Et_3N	OPh*m*-Cl	100	3.4
35	E	OH·Et_3N	OCH_2CH_2CN	41	>100
36	E	OH·Et_3N	OPh*m*-Cl	100	2.5
37	E	OH·Et_3N	Oph	100	6.7
38	E	OH·Et_3N	O*c*-hex	100	8.7

[a]Determined by the Stretch PCR assay.
[b]Inhibition with use of 100 μM of the ligand.

In conclusion, we have developed novel inhibitors for human telomerase. The most potent inhibitors (**34** and **36**) are constituted of two indole units, a long alkyl chain and a phosphodiester group. This novel structure will be useful as a scaffold to construct a compound library of telomerase inhibitors. Further study is now ongoing along this line for the construction of a new compound library.

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