Catecholic Flavonoids Acting as Telomerase Inhibitors

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In recent years telomerase has been identified as a new promising target in oncology and consequently new telomerase inhibitors have been intensely explored as anticancer agents. Focused screening of several polyhydroxylated flavonoids has allowed us to identify 7,8,3′,4′-tetrahydroxyflavone **1** as a new telomerase inhibitor with an interesting in vitro activity in a Flash-Plate assay ($IC_{50} = 0.2\ \mu M$) that has been confirmed in the classical TRAP assay. Starting from this compound, we developed a medicinal chemistry program to optimize our lead, and in particular to replace one of the two catechols with potential bioisosteres. From this study, new structural analogues characterized by submicromolar potencies have been obtained. Their synthesis and biological activity are described.

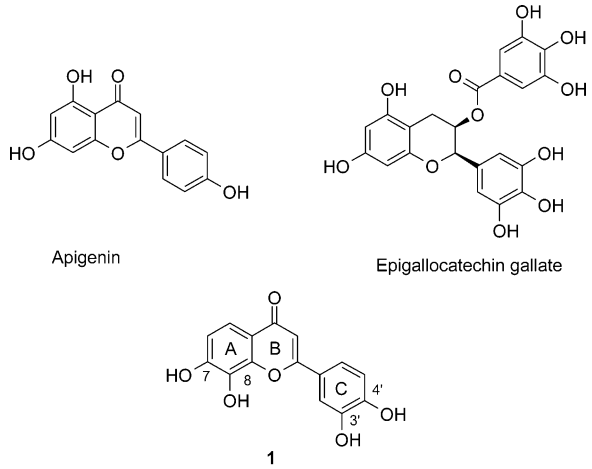
Introduction

The ends of chromosomes in higher eukaryotes are built up of telomeres, which consist of tandem arrays of hexameric TTAGGG sequences ranging from approximately 15 to 20 kb in normal human cells.[1] Telomeres protect the ends of chromosomes, and their stability and length are regulated by telomerase, which is a ribonucleoprotein responsible for adding telomere repeats to the 3′-ends. The enzyme consists of two components, an RNA, which serves as a template for the telomere repeats, and the catalytic subunit, which has reverse transcriptase activity. Telomeres shorten after each round of replication and serve as a "mitotic clock", which in normal cells limits the number of replications. This block can be bypassed in cancer cells by upregulation of telomerase, leading to unlimited proliferative capacity.[2,3] Telomerase is an attractive target for a potentially selective anticancer therapy due to its specific expression in tumors (>85%) compared to a low expression in normal somatic cells.[4]

Therefore, inhibition of telomerase has been approached through different strategies, ranging from antisense-based inhibitors to random screening of synthetic and natural products.[5,6] Among the latter, several compounds have been reported in the literature, including rubromycins,[7] alterperylenol,[8] diazaphilonic acid,[9] epigallocatechin gallate,[10] and apigenin.[11]

Flavonoids have been known for a long time to exert multiple biological effects (bioflavonoids), in particular to act as anticancer agents[12−14] and as inhibitors of other DNA polymerases like reverse transcriptases.[15,16] As already mentioned, examples of telomerase inhibitors within the flavonoid family have been described in the literature (epigallocatechin gallate and apigenin, Chart 1).

Chart 1. Structures of Flavonoid Leads



In particular the interesting activity of epigallocatechin gallate has been reconfirmed in our in vitro assay ($IC_{50} = 1\ \mu M$; for details on the assay, see below). For this reason we performed a focused screening of several polyhydroxylated compounds to identify new hits for telomerase inhibition. This effort, which took advantage of commercially available flavonoids, has resulted in the identification of the tetrahydroxy flavone **1** (Chart 1) with submicromolar potency in the in vitro assay ($IC_{50} = 0.2\ \mu M$). This compound is characterized by a biscatecholic substitution pattern that we argued to be probably essential for biological activity. On the other hand, these highly oxygenated functionalities were also thought to represent an intrinsic limitation for drug development. In fact. many flavonoids are often characterized by a labile nature due to their polyhydroxylated templates. Thus we started a medicinal chemistry program with the final goal to highlight the essential

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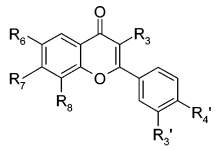
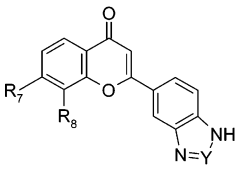


For **1a-f**, **2a-e** and **4a-b** $R_3 = R_6 = H$

$R_7 = R_8 = R_{4'} = OH$; $R_{3'} = H$	**1a**	$R_7 = R_8 = R_{3'} = R_{4'} = OMe$	**2a**
$R_7 = R_8 = OH$; $R_{3'} = R_{4'} = H$	**1b**	$R_7 = R_8 = OH$; $R_{3'} = R_{4'} = OMe$	**2b**
$R_7 = R_8 = R_{3'} = OH$; $R_{4'} = H$	**1c**	$R_7 = R_8 = OMe$; $R_{3'} = R_{4'} = OH$	**2c**
$R_7 = R_{3'} = R_{4'} = OH$; $R_8 = H$	**1d**	$R_7 = R_8 = R_{3'} = OH$; $R_{4'} = OMe$	**2d**
$R_7 = H$; $R_8 = R_{3'} = R_{4'} = OH$	**1e**	$R_7 = OMe$; $R_8 = R_{3'} = R_{4'} = OH$	**2e**
$R_7 = R_8 = H$; $R_{3'} = R_{4'} = OH$	**1f**		

$R_7 = R_8 = R_{3'} = R_{4'} = OH$; $R_3 = H$; $R_6 = Cl$	**3a**	$R_7 = R_8 = OMe$; $R_{3'} = R_{4'} = NH_2$	**4a**
" $R_3 = F$; $R_6 = H$	**3b**	$R_7 = R_8 = OMe$; $R_{3'} = R_{4'} = NHAc$	**4b**
" $R_3 = Cl$; $R_6 = H$	**3c**	$R_7 = R_8 = OH$; $R_{3'} = R_{4'} = NH_2$	**4c**
" $R_3 = CN$; $R_6 = H$	**3d**		

Figure 1. Structures of strictly related analogues of tetrahydroxyflavone **1**.



$R_7 = R_8 = OMe$; Y=CH	**4d**
$R_7 = R_8 = OH$; Y=CH	**4e**
$R_7 = R_8 = OMe$; Y=COH	**4f**
$R_7 = R_8 = OH$; Y=COH	**4g**
$R_7 = R_8 = OMe$; Y=CMe	**4h**
$R_7 = R_8 = OH$; Y=CMe	**4i**
$R_7 = R_8 = OMe$; $Y=C(CH_2)_3N(CH_3)_2$	**4j**
$R_7 = R_8 = OMe$; Y=CH(OH)CO	**4k**
$R_7 = R_8 = OH$; Y=CH(OH)CO	**4l**

Figure 2. Structures of bioisosteric catechol analogues of tetrahydroxyflavone **1**.

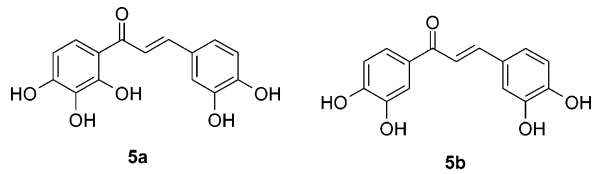


Figure 3. Structures of alternative scaffolds of tetrahydroxyflavone **1**.

structural features for the biological activity of flavone **1** and to identify alternative moieties to circumvent potentially unstable catechols.

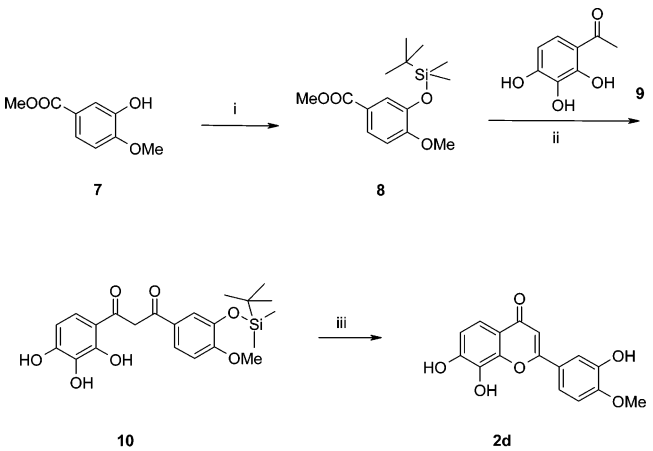
Biological results on compound **1** as well as the synthesis and the biological activity of the analogues prepared will be reported.

Chemistry

In Figures 1−3, chemical structures of analogues submitted to the in vitro telomerase inhibition assay are reported. Compounds **1a**−**1d**, **1f**, **2a**, and **5a** have been obtained from commercial sources. Compounds **1e**, **2b**, and **5b** have been prepared according to methods reported in the literature.[17−19]

Schemes 1 and 2 show the synthetic pathways leading to the target derivatives **2d** and **2e**,**c**. According to the literature,[18] reaction of the appropriate methyl O-silyloxylated benzoates **8** and **11** with the commercially available hydroxyacetophenones **9** and **12a**,**b** afforded the intermediate diketones **10** and **13a**,**b** that were subjected to cyclodehydration with 0.5% sulfuric acid in acetic acid at 100 °C. Under these reaction conditions, the *tert*-butyldimethylsilyl protecting groups were also cleaved to generate the desired partially methylated flavones **2d** and **2e**,**c**. In Scheme 3 the synthesis of flavone **3a** is reported. We have used the most common method of synthesizing flavones, known as the Baker−Venkataraman transformation.[20−22] In this process, an

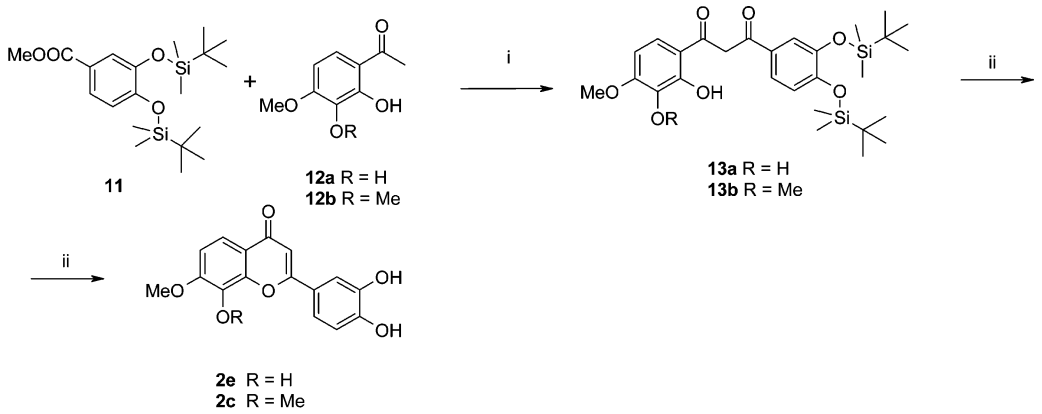
Scheme 1[a]



[a] Reagents: (i) $tBu(Me)_2SiCl$, DIEA, DMF, rt; (ii) LiHMDS, THF, −78 °C to rt; (iii) CH_3COOH, H_2SO_4, 100 °C.

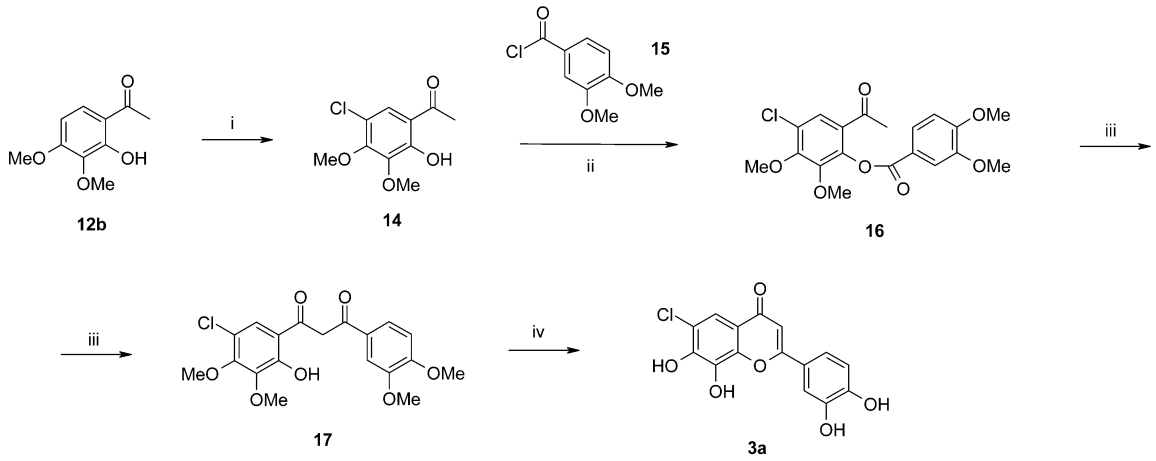
appropriate hydroxyacetophenone is first converted into a benzoyl ester that, after treatment with base, yields a 1,3-diketone. Treatment of the diketone with acid leads to the desired flavone. As reported in Scheme 3, the chloro derivative **14** was obtained by reaction of the commercially available 2,3-dimethoxy-2-hydroxyacetophenone (**12b**) with *N*-chlorosuccinimide in hot acetic acid.[23] Base-catalyzed condensation of **14** with the commercial 3,4-dimethoxybenzoyl chloride (**15**) furnished the intermediate benzoyl ester **16**, which was converted to the 1,3-diketone **17**. Contemporary cyclodehydration and demethylation with a mixture of hydriodic and acetic acids at reflux gave the final flavone **3a**.[24] The 3-substituted flavones **3b**−**d** were prepared according to the routes reported in Scheme 4. The common intermediate 1,3-diketone **19** was obtained likewise from the commercially available 2,3-dimethoxy-2-hydroxyacetophenone (**12b**) and 2,4-dimethoxybenzoyl chloride (**15**). Reaction of **19** with *N*-fluorodibenzosulfonimide gave intermediate **20**,[25] which was transformed by cyclodehydration under acidic conditions into 3-fluorotetramethoxy flavone **21**, which was converted to the corresponding tetrahydroxy derivative **3b** with boron tribromide.[26] Treatment of **19** with thionyl chloride in dioxane at reflux afforded the 3-chlorotetramethoxy flavone **22**,[27] which, after reaction with boron tribromide yielded derivative **3c**. Compound **22** was also used to prepare the 3-cyanotetramethoxy flavone **23**, in acceptable yield, by treatment with cuprous cyanide at 220 °C.[28] Subsequent demethylation with boron tribromide provided the final flavone **3d**. In Scheme 5 the synthesis of flavone **4b** is reported. Condensation of the acid chloride **24** with the commercially available 3,4-dimethoxy-2-hydroxyacetophenone (**12b**), in the presence of pyridine, furnished the benzoyl ester **25**. This was converted to the 1,3-diketone **26** by use of pyridine/KOH. Ring closure and amide hydrolysis of **26** with concentrated hydrochloric acid furnished flavone **28**.[29] Reduction of the nitro group by reaction with zinc and nickel(II) chloride hexahydrate in hot *N*,*N*-dimethylformamide (DMF) afforded the final compound **4b**. Flavone **4b** was also used as key intermediate for the synthesis of a series of desired products shown in Scheme 6. Demethylation of **4b** with hydrobromic acid at reflux gave the dihydroxy flavone **4c**,[30] while com-

Scheme 2[a]



[a] Reagents: (i) LiHMDS, THF, −78 °C to rt; (ii) CH_3COOH, H_2SO_4, 100 °C.

Scheme 3[a]



[a] Reagents: (i) NCS, CH_3COOH, 80 °C; (ii) pyridine, rt; (iii) KOH, pyridine, 50 °C; (iv) Hl, CH_3COOH, reflux.

pound **4a** was obtained, from **4b**, by use of acetyl chloride. The series of 2-[2-substituted-1*H*-benzimidazol-5(6)-yl]-4*H*-1-benzopyran-4-ones (**4d**,**h**,**j**) were achieved by a Phillips-type reaction from the corresponding aliphatic carboxylic acids (see Scheme 6). Demethylation of **4d**,**h** with hydrobromic acid at reflux afforded compounds **4e**,**i**. Treatment of **4b** with 1,1′-carbonyldiimidazole supplies the benzimidazole derivative **4f**,[32] while the use of 1,1′-oxalyldiimidazole instead of 1,1′-carbonyldiimidazole produced flavone **4k**.[33] The corresponding dihydroxy derivatives **4g**,**l** were achieved by reaction with hydrobromic acid at reflux.

Results and Discussion

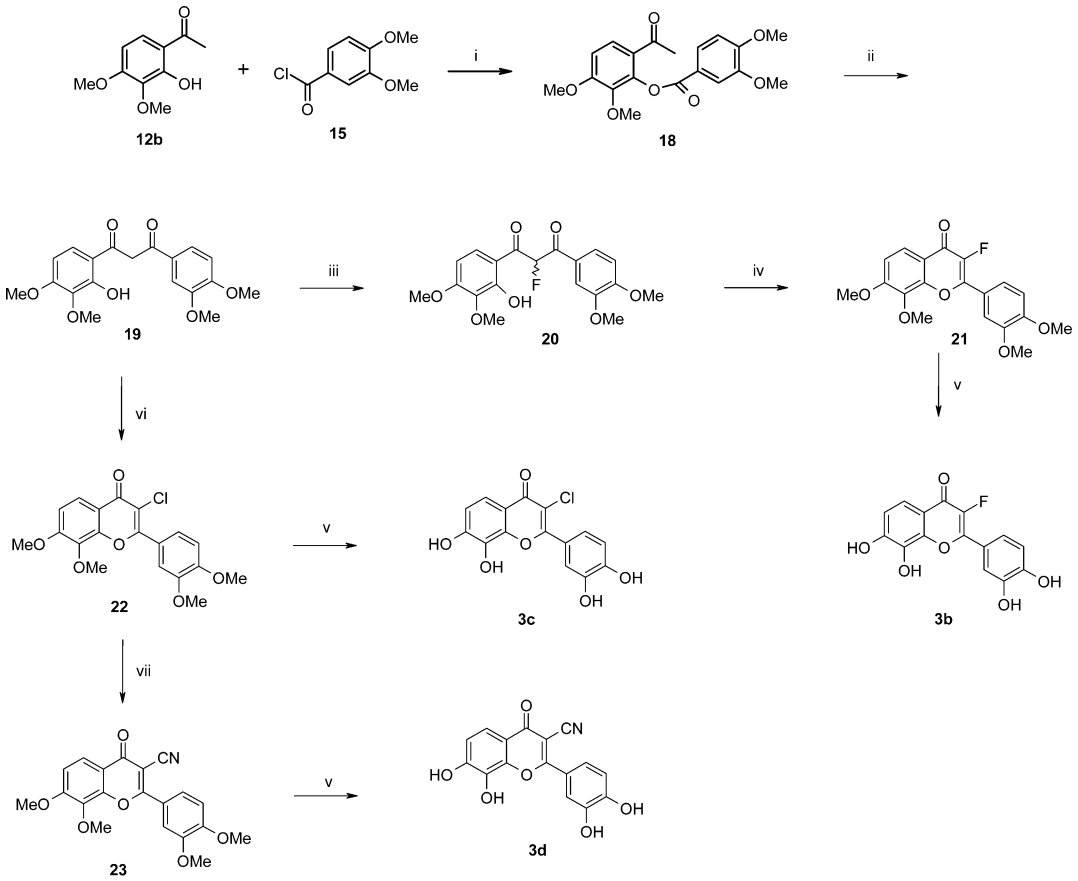
For the identification of telomerase inhibitors, an assay that quantifies the incorporation of nucleotides by telomerase in a microtiter format has been used, allowing high-throughput screening.[34] In particular, the elongation of a 5′-biotinylated primer in the presence of the corresponding dNTPs necessary for the telomeric sequence (dGTP, dATP, and TTP), was evaluated by hybridization in solution with a 3′-radiolabeled complementary oligonucleotide. The extent of hybridization was then quantitated by immobilizing the hybridized oligonucleotides in a streptavidin-coated Flash-Plate. Telomerase activity is proportional to the radioactivity measured, and the inhibitory activity of the compounds was evaluated as IC_{50} (see Experimental Section for details).

The tetrahydroxyflavone **1** has been recognized as a potent telomerase inhibitor in this Flash-Plate (FP) assay ($IC_{50} = 0.2\ \mu M$). In addition, compound **1** showed 50% inhibition at 1 μM also in the conventional polymerase chain reaction- (PCR-) based cell-free TRAP assay, with A431 cell lysates as a source of telomerase,[35,36] and in an intact cell assay, which determines the inhibition of intracellular telomerase activity (manuscript in preparation). Tetrahydroxyflavone **1** has also been tested in a selectivity assay, based on T3 RNA polymerase, to examine its specificcty of inhibition. In this assay it did not show inhibitory activity up to 2 μM.

These preliminary results on compound **1** triggered a more extensive investigation on this chemical class, and in particular in assessing the role of the hydroxyls in the enzymatic interaction and their replacement with more attractive substituents.

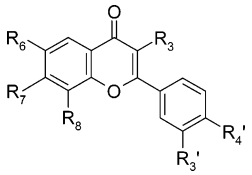
First of all, we considered a set of analogues with a reduced number of hydroxyls. As can be noticed from Table 1, the minimal structural requirement for activity is the simultaneous presence of hydroxyls at positions 7 and 8 (**1b**) (see numbering system in Chart 1). Improvement of activity can be achieved with additional hydroxyls on ring C, and in particular, low micromolar potency is ensured only if 4′-OH is present (**1a**), while the maximum of activity is reached with the biscatecholic pattern (**1**). On the contrary, when one of the two hydroxyls at position 7 or 8 is removed, a complete drop of activity is observed (**1d** and **1e**).

Scheme 4[a]



[a] Reagents: (i) pyridine, rt; (ii) KOH, pyridine, rt; (iii) $(PhSO_2)_2NF$, DCM, rt; (iv) CH_3COOH, H_2SO_4, 90 °C; (v) BBr_3, DCM, rt; (vi) $SOCl_2$, dioxane, reflux; (vii) CuCN, NMP, 220 °C.

Table 1. Flavone Substituents and Telomerase Inhibition Data (FP assay)



	R_7	R_8	R_3'	R_4'	R_3	R_6	IC_{50}[a] (μM)
1	OH	OH	OH	OH	H	H	0.2
1a	OH	OH	H	OH	H	H	3
1b	OH	OH	H	H	H	H	36
1c	OH	OH	OH	H	H	H	50
1d	OH	H	OH	OH	H	H	≫40
1e	H	OH	OH	OH	H	H	≫40
1f	H	H	OH	OH	H	H	≫40
2a	OCH_3	OCH_3	OCH_3	OCH_3	H	H	≫40
2b	OH	OH	OCH_3	OCH_3	H	H	11.7
2c	OCH_3	OCH_3	OH	OH	H	H	≫40
2d	OH	OH	OH	OCH_3	H	H	11
2e	OCH_3	OH	OH	OH	H	H	7.8
3a	OH	OH	OH	OH	H	Cl	0.82
3b	OH	OH	OH	OH	F	H	0.6
3c	OH	OH	OH	OH	Cl	H	0.8
3d	OH	OH	OH	OH	CN	H	0.13
4a	OCH_3	OCH_3	NH_2	NH_2	H	H	7.4
4b	OCH_3	OCH_3	NHAc	NHAc	H	H	≫40
4c	OH	OH	NH_2	NH_2	H	H	3.6

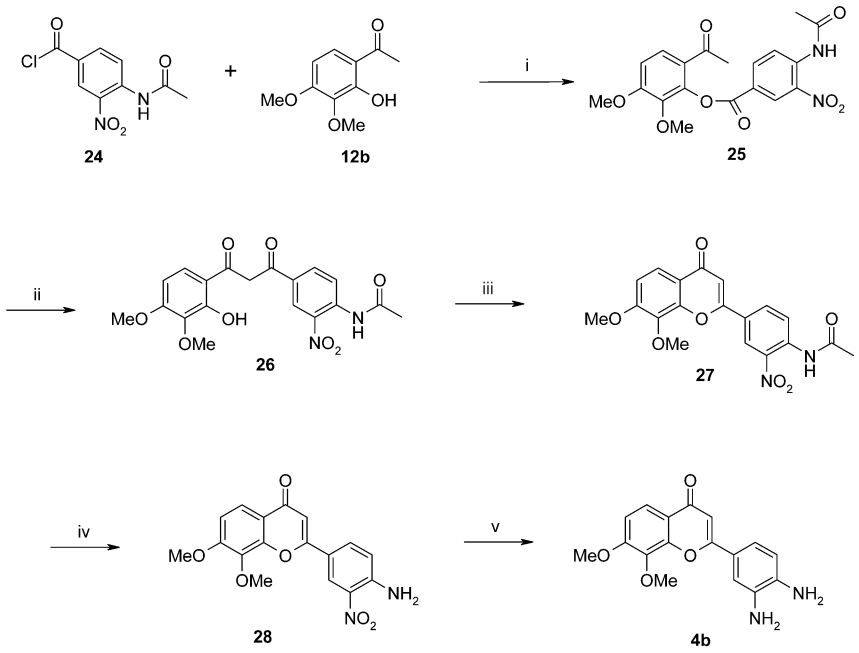
[a] Concentration required to inhibit telomerase activity by 50%.

Then we investigated the effect of partial or total methylation of the hydroxyl groups as a way to possibly prevent any oxidative degradation and, at the same time, to understand the importance of their H-bond donor/acceptor properties. As can be seen in Table 1, the tetramethoxy derivative is completely inactive (**2a**), showing the importance of free OH in the interaction with the enzyme. Compound **2b**, in which the catecholic moiety on ring A is restored, has shown moderate activity, while **2c**, where the catechol on ring C is present, is inactive, further confirming the importance for the activity of hydroxyls at position 7 and 8. Interestingly, when hydroxyl group at position 7 is methylated (compound **2e**), micromolar potency is observed. This result seems to suggest that the free OH at that position is required for optimal activity but not critical. The H-bond acceptor nature of the methoxy group can be also tolerated. Analogously, when only the 4′-OH is methylated (compound **2d**), activity in the micromolar range is observed.

Once the importance of the biscatechol for optimal activity was established, modulation of the electron density of this electron-rich template was a further object of our study. Various electron-withdrawing substituents (F, Cl, and CN) were introduced first onto position 3 (ring B, compounds **3b**−**d**, Table 1), thus interfering with the enone moiety, and then onto position 6 (Cl) (ring A, compound **3a**, Table 1), thus modifying the acidity of the proximal OH. Surprisingly, both sets of substitution did not affect sensibly the activity. This evidence suggests that the flavonoid skeleton might be not sensitive to modifications and might also be replaced by other scaffolds.

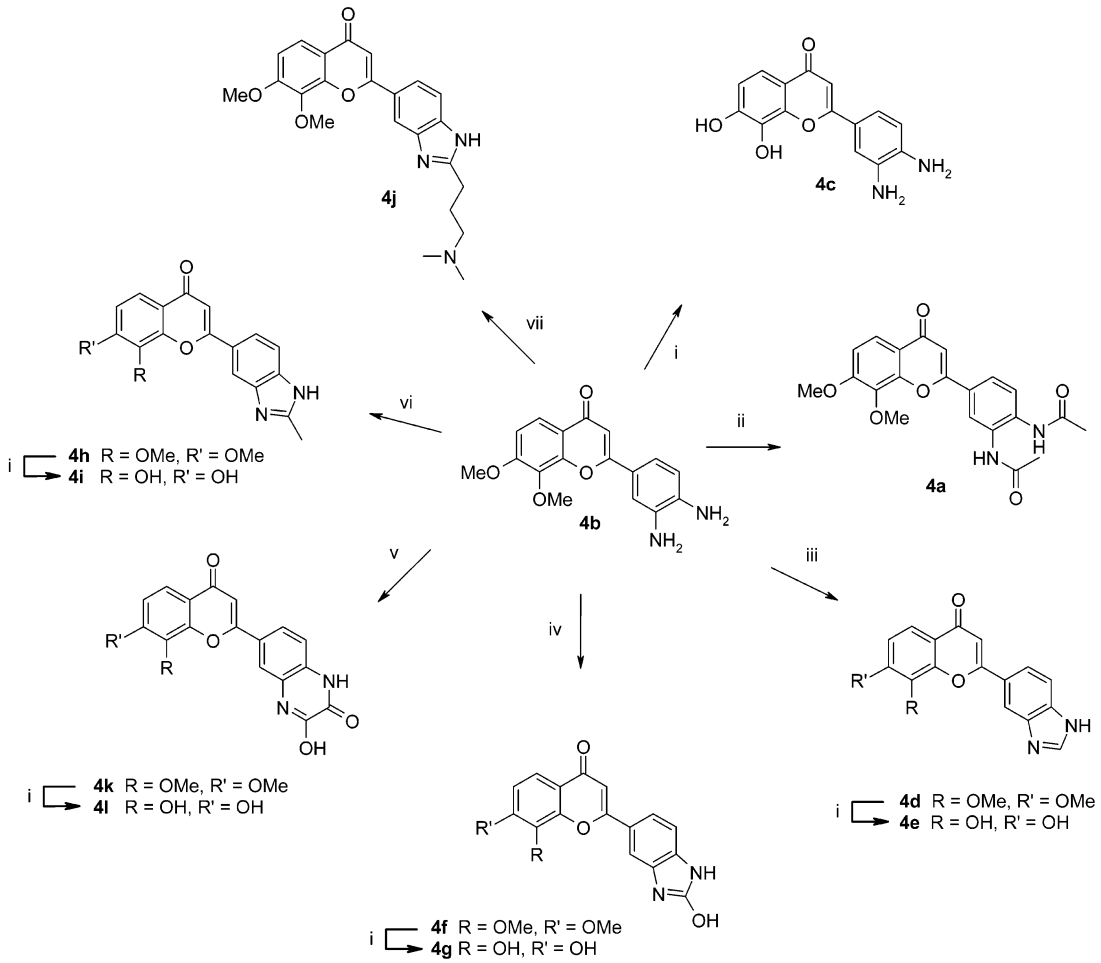
The next step involved modifications of both reactive catechol moieties. In particular, it was decided to replace

Scheme 5[a]



[a] Reagents: (i) pyridine, 0 °C to rt; (ii) KOH, pyridine, 60 °C; (iii) CH_3COOH, CH_3COONa, reflux; (iv) HCl, reflux; (v) Zn dust, $NiCl_2 \cdot 6H_2O$, DMF, MeOH, 70 °C.

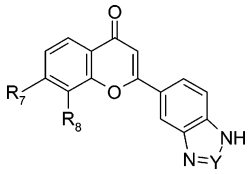
Scheme 6[a]



[a] Reagents: (i) 48% HBr, reflux; (ii) CH_3COCl, TEA, THF, 0 °C to rt; (iii) HCOOH/4 N HCl, reflux; (iv) 1,1′-carbonyldimidazole, THF, 0 °C to rt; (v) 1,1′-oxalyldiimidazole, DMF, rt; (vi) CH_3COOH/4 N HCl, reflux; (vii) $(CH_3)_2N(CH_2)_3COOH \cdot HCl$, 4 N HCl, reflux.

catechol on ring C first, as it proved to be less critical for the activity and thus possibly more flexible toward modifications. Our plan was to replace it with a convenient heterocyclic ring.

Table 2. Flavone Substituents and Telomerase Inhibition Data (FP Assay)



	R_7	R_8	Y	IC_{50}[a] (μM)
4d	OCH_3	OCH_3	CH	2.2
4e	OH	OH	CH	4
4f	OCH_3	OCH_3	COH	19
4g	OH	OH	COH	1.2
4h	OCH_3	OCH_3	CMe	22.1
4i	OH	OH	CMe	$\gg$40
4j	OCH_3	OCH_3	$C(CH_2)_3N(CH_3)_2$	0.47
4k	OCH_3	OCH_3	CH(OH)CO	$\gg$40
4l	OH	OH	CH(OH)CO	$\gg$40

[a] Concentration required to inhibit telomerase activity by 50%.

Bioisosteres of catechols have already been reported in the literature and they deal mainly with nitrogen-containing heterocyclic derivatives. For example, novel ligands of dopamine receptors have been identified by replacing the catechol moiety of dopamine by benzimidazole-2-thione and 2,3-dihydroquinoxaline.[37] Analogously, benzimidazole derivatives of catecholamines succeeded in showing agonistic activity at the adrenergic receptors, thus confirming the bioisosteric role of benzimidazole with respect to catechol.[38]

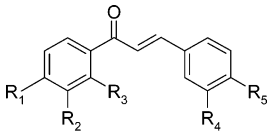
Therefore, we focused our attention on the imidazole ring, both unsubstituted and substituted at position 2, and on the piperazinedione ring, as reported in Table 2 (compounds **4d**−**l**). The corresponding precursors are compounds **4a**−**c**, reported in Table 1. Compound **4a**, the 3′,4′ diamino analogue of **1** protected as dimethoxy at positions 7−8, and the fully deprotected **4c** were surprisingly almost equally active in the micromolar range, while the diacetyl derivative **4b** turned out to be inactive, as expected.

Analogously, compounds **4d** and **4e**, bearing an unsubstituted imidazole at ring C, are almost equally active. Instead, when oxygen was introduced at position 2 of the imidazole ring, dimethoxy derivative **4f** turned out to be 1 order of magnitude less active than the corresponding dihydroxy **4g**, consistent with the hypothesis that the catechol on ring A is more important for a good enzymatic interaction. However the insertion of alkylic substituents on the imidazole completely changes the activity trend. In fact, in the case of methyl, compound **4h** (7,8-dimethoxy derivative) shows a weak potency, while the dihydroxy **4i** is completely inactive.

Despite the lack of information on the mode of action of these compounds, we were interested in checking the possibility of an interaction with the DNA polyanionic backbone through a group charged at physiological pH. Thus we introduced at position 2 of imidazole the short dimethylaminopropyl chain (compound **4j**), present in some G-quadruplex interacting compounds reported in the literature.[39−41] Interestingly, derivative **4j** showed a very good potency compared to its parent compound **4d**, suggesting some additional interactions with the enzyme. This result supports our hypothesis, and further studies are currently in progress.

Finally, piperazinedione insertion on ring C resulted in a complete lack of activity, for both dimethoxy and dihydroxy derivatives (compounds **4k** and **4l**).

Table 3. Chalcone Substituents and Telomerase Inhibition Data (FP Assay)



	R_1	R_2	R_3	R_4	R_5	IC_{50}[a] (μM)
5a	OH	OH	OH	OH	OH	6
5b	OH	OH	H	OH	OH	1.7

[a] Concentration required to inhibit telomerase activity by 50%.

The effect of more flexible scaffolds on the activity was also studied by opening the benzopyranone, thus giving rise to chalcones (Table 3, **5a,b**). Both compounds are weaker inhibitors with respect to their cyclic parent compound **1**, the best one (**5b**) being almost 10-fold less active. This result suggests a preference for cyclic templates.

Conclusion

In conclusion, although the complete lack of structural information on the enzymatic target has hampered a full understanding of drug interaction at the active site and thus a rational approach to analogue design, new telomerase inhibitors have been discovered by the use of a Flash-Plate assay suitable for high-throughput screening. In fact we have identified a new telomerase inhibitor with a catecholic flavonoid structure (compound **1**) endowed with an interesting activity profile. Medicinal chemistry studies on this lead compound allowed the understanding of bioactivity determinants. In addition, preparation of new catecholic derivatives still endowed with submicromolar potencies has been achieved (compounds **3a**−**d**).

Finally, replacement of catechol on ring C with bioisosteric substitutents yielded the analogue **4j** characterized by submicromolar potency and improved chemical profile. It represents a new lead structure to be considered for further investigation.

Experimental Section

^{1}H NMR spectra were recorded with a Varian 300 or 400 spectrometer. Chemical shifts are reported as δ values (parts per million, ppm) downfield from internal Me_4Si in the solvent shown. The following NMR abbreviations are used: br (broad), s (singlet), d (doublet), t (triplet), m (multiplet), ex (exchangeable with D_2O). Other abbreviations: ESI, electrospray ionization; MS, mass spectrometry; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. ESI MS spectra were recorded on a ion-trap mass LCQ-Deca Finningan spectrometer. Elemental analyses were performed on a EA 1110 CHNS−O C. E. Instruments elemental analyzer and agreed with theoretical values to within ±0.4%. Compounds **1**, **1a,b**, **1d**, and **2a** were purchased from Apin Chemicals Ltd. Compound **1c** was purchased from Indofine Chemical Co, Ltd., and compound **1f** was from Lancaster Synthesis Ltd.

T3 RNA polymerase inhibition has been evaluated by using the T3 RNA polymerase kit (Stratagene) and adapting the instructions of kit.

The conventional PCR-based cell-free TRAP assay has been run by using TRAP-eze kit (ONCOR) according to the instructions of the manufacturer.

Methyl 3-{[*tert*-Butyl(dimethyl)silyl]oxy}-4-methoxybenzoate (8). To a solution of methyl 3-hydroxy-4-methoxybenzoate (1.5 g, 8.2 mmol) in dry DMF (20 mL), under argon

at 0 °C, *N*-ethyldiisopropylamine (2.8 mL, 16.4 mmol) is added dropwise, followed by a solution of *tert*-butylchlorodimethylsilane (1.55 g, 10.3 mmol) in dry DMF (10 mL). The reaction mixture is stirred at room temperature overnight, iced water is added, and the precipitate is filtered, washed with water, and dried to yield **8** as a white powder (98%). ^{1}H NMR (DMSO-d_6) δ 0.12 (6H, s), 0.95 (9H, s), 3.80 (3H, s), 3.84 (3H, s), 7.08 (1H, d), 7.33 (1H, s), 7.60 (1H, d). MS (ESI) *m/z* 297 [(M + H)$^+$].

1-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-4-methoxyphenyl)-3-(2,3,4-trihydroxyphenyl)-1,3-propanedione (10). To a solution of 2,3,4-trihydroxyacetophenone (0.5 g, 2.97 mmol) in dry THF (20 mL), cooled to −78 °C under argon, lithium bis(trimethylsilyl)amide (1M solution in THF, 14.8 mL) is added dropwise in 15 min, and the solution is stirred at −78 °C for 1 h and at −10 °C for 2 h. To the mixture, cooled to −78 °C, a solution of **8** (0.88 g, 2.97 mmol) in dry THF (5 mL) is added dropwise, and the reaction mixture is stirred at −78 °C for 1 h and at room temperature overnight. The mixture is poured into ice, 20% aqueous HCl (5 mL) is added (pH 2.5), and the precipitate is extracted with ethyl acetate. The organic layer is separated and washed with brine, dried, and concentrated to yield dark oil that is stirred in isopropyl ether/hexane (1:1) and filtered. The solid is washed with hexane and dried to obtain **10** (45%). ^{1}H NMR (DMSO-d_6) δ 0.17 (6H, s), 1.01 (9H, s), 3.88 (3H, s), 4.53 (2H, s), 6.56 (1H, d), 7.05 (1H, d), 7.18 (1H, d), 7.62 (1H, d), 7.84 (1H, d), 10.41 (3H, br s, ex). MS (ESI) *m/z* 433 [(M + H)$^+$].

By analogous procedures, compounds **13a**,**b** were prepared:

1-(3,4-Di-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-(2,3-dihydroxy-4-methoxyphenyl)-1,3-propanedione (13a). Yield 75%. ^{1}H NMR (DMSO-d_6) δ 0.25 (12H, s), 1.02 (18H, s), 3.76 (3H, s), 4.50 (2H, s), 6.77 (1H, d), 7.10−7.16 (2H, m), 7.41 (2H, br s, ex), 7.60 (1H, d), 7.72 (1H, d). MS (ESI) *m/z* 547 [(M + H)$^+$].

1-(3,4-Di-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-3-(2-hydroxy-3,4-dimethoxyphenyl)-1,3-propanedione (13b). Yield 80%. ^{1}H NMR (DMSO-d_6) δ 0.25 (12H, s), 1.04 (18H, s), 3.86 (3H, s), 3.96 (3H, s), 4.53 (2H, s), 6.63 (1H, d), 7.12−7.18 (2H, m), 7.63 (1H, d), 7.75 (1H, d), 10.90 (1H, br s, ex). MS (ESI) *m/z* 561 [(M + H)$^+$].

7,8-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-chromen-4-one (2d). Compound **10** is dissolved in glacial acetic acid (10 mL), 96% sulfuric acid (0.05 mL) is added, and the solution is stirred at 100 °C for 1 h. The mixture is poured into ice and the precipitate is extracted with ethyl acetate. The organic layer is separated and washed with brine, dried over sodium sulfate, and concentrated to yield **2d** as a dark solid that is crystallized from dichloromethane/methanol (42%). ^{1}H NMR (DMSO-d_6) δ 3.29 (3H, s), 6.63 (1H, s), 6.91(1H, d), 7.07 (1H, d), 7.36 (1H, d), 7.50 (1H, d), 9.35 (1H, s), 9.38 (1H, s), 10.26 (1H, s). MS (ESI) *m/z* 301 [(M + H)$^+$]. Anal. ($C_{16}H_{12}O_6$) C, H.

By analogous procedures, compounds **2e** and **2c** were prepared:

8-Hydroxy-7-methoxy-2-(3,4-dihydroxyphenyl)-4*H*-chromen-4-one (2e). Yield 63%. ^{1}H NMR (DMSO-d_6) δ 3.93 (3H, s), 6.60 (1H, s), 6.87 (1H, d), 7.15 (1H, d), 7.40−7.50 (3H, m), 9.38 (1H, br s, ex), 9.55 (1H, br s, ex), 9.75 (1H, br s, ex). MS (ESI) *m/z* 301 [(M + H)$^+$]. Anal. ($C_{16}H_{12}O_6$) C, H.

7,8-Dimethoxy-2-(3,4-dihydroxyphenyl)-4*H*-chromen-4-one (2c). Yield 86%. ^{1}H NMR (DMSO-d_6) δ 3.94 (6H, s), 6.65 (1H, s), 6.89 (1H, d), 7.23 (1H, d), 7.39 (1H, d), 7.43 (1H, d), 7.73 (1H, d), 9.40 (1H, br s, ex), 9.80 (1H, br s, ex). MS (ESI) *m/z* 315 [(M + H)$^+$]. Anal. ($C_{17}H_{14}O_6$) C, H.

1-(5-Chloro-2-hydroxy-3,4-dimethoxyphenyl)ethanone (14). A solution of 3,4-dimethoxy-2-hydroxyacetophenone (0.2 g, 1.02 mmol) and *N*-chlorosuccinimide (0.16 g, 1.22 mmol) in glacial acetic acid (5 mL) is stirred at 80 °C for 6 h. After cooling, the solution is diluted with water and extracted with ethyl acetate. The organic phase is washed with water and brine, dried, and evaporated under reduced pressure. The crude reaction product is purified by flash chromatography (eluant, hexane/ethyl acetate 9:1) to yield **14** as a yellowish solid (61%). ^{1}H NMR ($CDCl_3$) δ 2.59 (3H, s), 3.91 (3H, s), 4.08 (3H, s), 7.52 (1H, s). MS (ESI) *m/z* 231 [(M + H)$^+$].

6-Acetyl-4-chloro-2,3-dimethoxyphenyl 3,4-dimethoxybenzoate (16). To a solution of **14** (0.4 g, 1.73 mmol) in anhydrous pyridine (5 mL), under argon, 3,4-dimethoxybenzoyl chloride (0.52 g, 2.59 mmol) is added in a period of 15 min. The mixture is stirred for 2 h at room temperature and then acidified with 2 N HCl, extracted with ethyl acetate, washed with water, dried, and evaporated under reduced pressure. The crude reaction product is purified by flash chromatography (eluant, hexane/ethyl acetate 7:3) to yield **16** as white solid (88%). ^{1}H NMR (DMSO-d_6) δ 2.45 (3H, s), 3.78 (3H, s), 3.82 (3H, s), 3.88 (3H, s), 3.92 (3H, s), 7.18 (1H, d), 7.55 (1H, d), 7.78−7.82 (2H, m). MS (ESI) *m/z* 395 [(M + H)$^+$].

By analogous procedures and starting from the properly substituted ethanones and aroyl chlorides, the following compounds were prepared:

6-Acetyl-2,3-dimethoxyphenyl-3,4-dimethoxybenzoate (18). Yield 80%. ^{1}H NMR (DMSO-d_6) δ 2.42 (3H, s), 3.68 (3H, s), 3.83 (3H, s), 3.87 (3H, s), 3.92 (3H, s), 7.11 (1H, d), 7.13 (1H, d), 7.55 (1H, d), 7.71 (1H, d), 7.78 (1H, d). MS (ESI) *m/z* 361 [(M + H)$^+$].

6-Acetyl-2,3-dimethoxyphenyl-4-(acetylamino)-3-nitrobenzoate (25). Yield 78%. ^{1}H NMR ($CDCl_3$) δ 2.35 (3H, s), 2.45 (3H, s), 3.80 (3H, s), 3.95 (3H, s), 6.90 (1H, d), 7.65 (1H, d), 8.43 (1H, d), 9.00 (1H, d), 9.10 (1H, s), 10.60 (1H, br s, ex). MS (ESI) *m/z* 403 [(M + H)$^+$].

1-(5-Chloro-2-hydroxy-3,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1,3-propanedione (17). To a solution of 6-acetyl-4-chloro-2,3-dimethoxyphenyl-3,4-dimethoxybenzoate (16) (0.58 g, 1.47 mmol) in anhydrous pyridine (5 mL), stirred at 50 °C, powdered potassium hydroxide (0.12 g, 2.25 mmol) is added. After 1 h, the reaction mixture is cooled, acidified with 2 N HCl, extracted with ethyl acetate, washed with water, dried, and evaporated under reduced pressure. The crude reaction product is purified by flash chromatography (eluant hexane/ethyl acetate 7:3) to yield **17** as yellow solid (62%). ^{1}H NMR (DMSO-d_6) δ 3.80 (3H, s), 3.82−3.90 (6H, m), 3.88 (3H, s), 7.10 (1H, d), 7.25 (1H, s), 7.55 (1H, d), 7.75 (1H, d), 7.95 (1H, s), 11.40−11.60 (2H, m, ex). MS (ESI) *m/z* 395 [(M + H)$^+$].

By analogous procedures and starting from the appropriate aryl benzoates, the following compounds were prepared:

1-(3,4-Dimethoxyphenyl)-3-(2-hydroxy-3,4-dimethoxyphenyl)-1,3-propanedione (19). Yield 78%. ^{1}H NMR (DMSO-d_6) δ 3.60−4.00 (12H, m), 4.75 (2H, s), 6.70 (1H, m), 7.00 (1H, m), 7.20 (1H, s), 7.70 (1H, m), 11.80 (1H, s, ex). MS (ESI) *m/z* 361 [(M + H)$^+$].

***N*-{4-[3-(2-Hydroxy-3,4-dimethoxyphenyl)-3-oxopropanoyl]-2-nitrophenyl}acetamide (26).** Yield 65%. ^{1}H NMR ($CDCl_3$) δ 2.38 (3H, s), 3.90 (3H, s), 3.98 (3H, s), 6.60 (1H, d), 6.78 (1H, s), 7.60 (1H, d), 8.18 (1H, d), 8.80 (1H, d), 9.00 (1H, d), 10.55 (1H, br s, ex), 11.50 (1H, br s, ex). MS (ESI) *m/z* 403 [(M + H)$^+$].

6-Chloro-2-(3,4-dihydroxyphenyl)-7,8-dihydroxy-4*H*-chromen-4-one (3a) A suspension of 6-chloro-2-(3,4-dimethoxyphenyl)-7,8-dimethoxy-4*H*-chromen-4-one (**17**) (0.11 g, 0.29 mmol) in a mixture of aqueous hydriodic acid (57%, 4 mL) and glacial acetic acid (4 mL) is refluxed for 15 h. After cooling, the yellow precipitate is filtered and washed with acetic acid and water. The solid is suspended into an aqueous $NaHSO_3$ solution and extracted with 1-butanol. The organic phase is washed with water, dried, and evaporated under reduced pressure. The crude product is washed with hot absolute ethanol and ether and dried in a vacuum to yield **3a** as a yellow solid (38%). ^{1}H NMR (DMSO-d_6) δ 6.61 (1H, s), 6.87 (1H, d,), 7.43 (1H, s), 7.50−7.55 (2H, m). MS (ESI) *m/z* 321 [(M + H)$^+$]. Anal. ($C_{15}H_9ClO_6$) C, H.

1-(3,4-Dimethoxyphenyl)-2-fluoro-3-(2-hydroxy-3,4-dimethoxyphenyl)-1,3-propanedione (20). To a solution of 1-(3,4-dimethoxyphenyl)-3-(2-hydroxy-3,4-dimethoxyphenyl)-1,3-propanedione (19) (0.36 g, 1 mmol) in anhydrous dichloromethane (20 mL), under argon, a solution of *N*-fluorodibenzosulfonimide (0.41 g, 1.3 mmol) in anhydrous dichloromethane

(20 mL) is added, and the solution is stirred at room temperature for 7 days. After solvent removal, the crude product is purified by flash chromatography (eluant, dichloromethane/methanol 100:1), to obtain **20** (28%). ^{1}H NMR (DMSO-d_6) δ 3.73 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 5.70 (1H, d), 6.80 (1H, d), 7.04 (1H, d), 7.23 (1H, d), 7.27 (1H, d), 7.52 (1H, d), 8.07 (1H, s). MS (ESI) *m/z* 379 [(M + H)$^+$].

2-(3,4-Dimethoxyphenyl)-3-fluoro-7,8-dimethoxy-4*H*-chromen-4-one (21). Product **20** (0.02 g, 0.05 mmol) is dissolved in glacial acetic acid (1 mL), 96% H_2SO_4 (0.01 mL) is added, and the solution is refluxed for 1 h. Water (10 mL) is added, and the precipitate is filtered, washed to neutrality, and dried to yield **21** (82%). ^{1}H NMR (DMSO-d_6) δ 3.85 (3H, s), 3.86 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 7.23 (1H, d), 7.32 (1H, d), 7.51 (1H, d), 7.60 (1H, d), 7.83 (1H, d). MS (ESI) *m/z* 361 [(M + H)$^+$]. Anal. ($C_{19}H_{17}FO_6$) C, H.

3-Chloro-2-(3,4-dimethoxyphenyl)-7,8-dimethoxy-4*H*-chromen-4-one (22). A portion of 1-(3,4-dimethoxyphenyl)-3-(2-hydroxy-3,4-dimethoxyphenyl)-1,3-propanedione (**19**) (1 g, 2.77 mmol) is dissolved in dry dioxane (20 mL), sulfuryl chloride (0.25 mL, 3.1 mmol) is added, and the mixture is refluxed for 1 h. The mixture is cooled to room temperature. (white precipitate), iced water is added (70 mL), and the precipitate is filtered, washed thoroughly with water, and dried. The solid is stirred in isopropyl ether (2 × 40 mL) and filtered. After crystallization from dichloromethane/isopropyl ether, **22**, as a white solid, is obtained (77%). ^{1}H NMR (DMSO-d_6) δ 3.81 (3H, s), 3.86 (3H, s), 3.89 (3H, s), 3.95 (3H, s), 7.18 (1H, d), 7.32 (1H, d), 7.50 (1H, d), 7.55 (1H, d), 7.82 (1H, d). MS (ESI) *m/z* 377 [(M + H)$^+$]. Anal. ($C_{19}H_{17}ClO_6$) C, H.

3-Cyano-2-(3,4-dimethoxyphenyl)-7,8-dimethoxy-4*H*-chromen-4-one (23). Compound **22** (0.38 g, 1 mmol) is suspended in 1-methyl-2-pyrrolidinone (5 mL), cuprous cyanide (0.16 g, 1.8 mmol) is added, and the mixture is refluxed overnight. The solvent is evaporated under vacuum and the crude material is purified by flash chromatography (eluant, dichloromethane/ethyl acetate 95:5) to yield **23** (54%). ^{1}H NMR (DMSO-d_6) δ 3.90−4.00 (12H, m), 7.30 (1H, d), 7.45 (1H, d), 7.65 (1H, d), 7.75 (1H, d), 7.80 (1H, d). MS (ESI) *m/z* 368 [(M + H)$^+$]. Anal. ($C_{20}H_{17}NO_6$) C, H, N.

3-Chloro-2-(3,4-dihydroxyphenyl)-7,8-dihydroxy-4*H*-chromen-4-one (3c). To a portion (0.15 g, 0.398 mmol) of 3-chloro-2-(3,4-dimethoxyphenyl)-7,8-dimethoxy-4*H*-chromen-4-one (22), dissolved in anhydrous dichloromethane (15 mL) and cooled to 0 °C, a 1 M solution of BBr_3 in dichloromethane (4.8 mL) is dropped slowly; the solution is stirred at room temperature for 2 h and then is diluted with iced water. The pH is arranged to 6 with 5% Na_2HPO_4, the mixture is extracted with ethyl acetate, and the organic layer is separated and washed with brine, dried, and concentrated to yield, after crystallization from dichloromethane/methanol/ethyl acetate, **3c** as yellow solid (42%). ^{1}H NMR (DMSO-d_6) δ 6.90 (1H, d), 6.97 (1H, d), 7.31 (1H, d), 7.38 (1H, d), 7.42 (1H, d), 9.22 (1H, s), 9.35 (1H, s), 9.80 (1H, s), 10.60 (1H, s). MS (ESI) *m/z* 321 [(M + H)$^+$]. Anal. ($C_{15}H_9ClO_6$) C, H.

By analogous procedures and starting from the appropriate precursors **21**, **23**, **31**, and **34**, the corresponding following compounds were prepared:

3-Fluoro-2-(3,4-dihydroxyphenyl)-7,8-dihydroxy-4*H*-chromen-4-one (3b). Yield 71%. ^{1}H NMR (DMSO-d_6) δ 6.90−7.01 (2H, m), 7.41−7.52 (3H, m), 9.45−9.46 (2H, m. ex), 9.79 (1H, s, ex), 10.44 (1H, s, ex). MS (ESI) *m/z* 305 [(M + H)$^+$]. Anal. ($C_{15}H_9FO_6$) C, H.

3-Cyano-2-(3,4-dihydroxyphenyl)-7,8-dihydroxy-4*H*-chromen-4-one (3d). Yield 56%. ^{1}H NMR (DMSO-d_6) δ 6.94−7.04 (2H, m), 7.41 (1H, d), 7.50−7.60 (2H, m), 9.43 (1H, s, ex), 9.47 (1H, s, ex), 9.85 (1H, s, ex), 10.80 (1H, s, ex). MS (ESI) *m/z* 312 [(M + H)$^+$]. Anal. ($C_{16}H_9NO_6$) C, H, N.

***N*-[4-(7,8-Dimethoxy-4-oxo-4*H*-chromen-2-yl)-2-nitrophenyl]acetamide (27).** A suspension of *N*-{4-[3-(2-hydroxy-3,4-dimethoxyphenyl)-3-oxopropanoyl]-2-nitrophenyl}acetamide (**26**) (0.25 g, 0.62 mmol) and sodium acetate (0.5 g) in glacial acetic acid (5 mL) is refluxed for 2 h. After cooling, the precipitate is filtered, washed with acetic acid, water, and ethyl acetate, and dried to yield **27** as a yellow solid (67%). ^{1}H NMR (DMSO-d_6) δ 2.10 (3H, s), 3.95−3.97 (6H, m), 7.08 (1H, s), 7.30 (1H, d), 7.80 (1H, d), 7.90 (1H, d), 8.35 (1H, d), 8.55 (1H, d), 10.50 (1H, br s, ex). MS (ESI) *m/z* 385 [(M + H)$^+$].

2-(4-Amino-3-nitrophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (28). A suspension of **27** (0.2 g, 0.52 mmol) in 9 N HCl (5 mL) is refluxed for 2 h. After cooling, the precipitate is filtered, washed with water, methanol, and ether, and dried in a vacuum to give **28** as a yellow solid (92%). ^{1}H NMR (DMSO-d_6) δ 3.95−3.97 (6H, m), 6.80 (1H, s), 7.15 (1H, d), 7.25 (1H, d), 7.75 (1H, d), 7.95 (1H, br s, ex), 8.05 (1H, d), 8.65 (1H, d). MS (ESI) *m/z* 343 [(M + H)$^+$].

2-(3,4-Diaminophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (4b). Product **28** (0.1 g, 0.29 mmol) and $NiCl_2 \cdot 6H_2O$ (0.14 g, 0.58 mmol) are suspended in a mixture of methanol/DMF (4:1, 5 mL), and Zn powder (0.15 g, 2.32 mmol) is added in portions with stirring. The solution is then heated at 70 °C for 2 h. The precipitate is separated by filtration while hot and washed with methanol. The filtrate and the washing are combined and the solvent is evaporated under reduced pressure. The crude product is then suspended in a mixture of methanol/water (8:2, 10 mL), stirred for 30 min, filtered, washed with water, and dried in a vacuum to yield **4b** as a yellow solid (79%). ^{1}H NMR (DMSO-d_6) δ 3.95−3.97 (6H, m), 4.80 (2H, br s), 5.35 (2H, br s, ex), 6.45 (1H, s), 6.60 (1H, d), 7.15−7.25 (3H, m), 7.70 (1H, d). MS (ESI) *m/z* 313 [(M + H)$^+$]. Anal. ($C_{17}H_{16}N_2O_4$) C, H, N.

***N*-[2-(Acetylamino)-4-(7,8-dimethoxy-4-oxo-4*H*-chromen-2-*yl*)phenyl]acetamide (4a).** A suspension of **4b** (0.15 g, 0.48 mmol) and trietylamine (0.53 mL, 3.84 mmol) in dry tetrahydrofuran (3 mL) is cooled (0 °C), and acetyl chloride (0.14 mL, 1.92 mmol) is added with stirring. The cooling bath is removed and stirring is continued overnight. The reaction mixture is then filtered and the solid is washed with tetrahydrofuran and dried. The crude product is suspended in water (15 mL), stirred at room temperature for 30 min, filtered, washed with water, and dried in a vacuum at 50 °C to yield **4a** as a yellow solid (33%). ^{1}H NMR (DMSO-d_6) δ 2.05 (3H, s), 2.10 (3H, s), 3.95−3.98 (6H, m), 6.80 (1H, s), 7.25 (1H, d), 7.75 (1H, d), 7.80−7.90 (2H, m), 8.30 (1H, s), 9.50−9.52 (2H, m, ex). MS (ESI) *m/z* 397 [(M + H)$^+$]. Anal. ($C_{21}H_{20}N_2O_6$) C, H, N.

2-(1*H*-Benzimidazol-5-yl)-7,8-dimethoxy-4*H*-chromen-4-one (4d). A suspension of 2-(3,4-diaminophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (**4b**) (0.2 g, 0.64 mmol) in a mixture of 4 N HCl (5 mL) and formic acid (1 mL) is heated at 100 °C for 2 h. After cooling, the reaction mixture is carefully neutralized with sodium bicarbonate powder and the solid precipitated is filtered, washed with water, methanol, and ether, and dried in a vacuum to obtain **4d** as a white solid (77%). ^{1}H NMR (DMSO-d_6) δ 3.96 (3H, s), 3.98 (3H, s), 6.95 (1H, s), 7.25 (1H, d), 7.70−8.00 (4H, m), 8.40 (1H, s), 11.90 (1H, br s, ex). MS (ESI) *m/z* 323 [(M + H)$^+$]. Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

2-(2-Hydroxy-1*H*-benzimidazol-5-yl)-7,8-dimethoxy-4*H*-chromen-4-one (4f). A suspension of 2-(3,4-diaminophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (**4b**) (0.1 g, 0.32 mmol) in dry tetrahydrofuran (5 mL) is cooled (0 °C) and *N*,*N*′-carbonyldiimidazole (0.062 g, 0.38 mmol) is added rapidly with stirring. The cooling bath is removed and stirring is continued overnight. The reaction mixture is then filtered and the solid is washed with tetrahydrofuran, methanol, and ether and dried in a vacuum to yield **4f** as a yellow solid (80%). ^{1}H NMR (DMSO-d_6) δ 3.95−3.98 (6H, m), 6.80 (1H, s), 7.10 (1H, d), 7.25 (1H, d), 7.55 (1H, d), 7.70 (1H, d), 7.80 (1H, d), 10.90 (1H, br s, ex), 11.0 (1H, br s, ex). MS (ESI) *m/z* 339 [(M + H)$^+$]. Anal. ($C_{18}H_{14}N_2O_5$) C, H, N.

7,8-Dimethoxy-2-(2-methyl-1*H*-benzimidazol-5-yl)-4*H*-chromen-4-one (4h). A suspension of 2-(3,4-diaminophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (**4b**) (0.5 g, 1.60 mmol) in a mixture of 4 N HCl (5 mL) and glacial acetic acid (2.5 mL) is heated at 100 °C for 2 h. After cooling, the reaction mixture is carefully neutralized with sodium bicarbonate powder and the solid precipitated is extracted with chloroform (3 × 50 mL). The organic phase is dried, filtered, and evaporated. The crude product is purified by use of boiling methanol to obtain pure

4h (43%). ^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 3.95−4.00 (6H, m), 6.80 (1H, s), 6.95 (1H, s), 7.25 (1H, d), 7.60 (1H, br s), 7.80 (1H, d), 7.85 (1H, d), 8.20 (1H, br s, ex). MS (ESI) *m/z* 337 [(M + H)$^+$]. Anal. ($C_{19}H_{16}N_2O_4$) C, H, N.

2-{2-[3-(Dimethylamino)propyl]-1*H*-benzimidazol-5-yl}-7,8-dimethoxy-4*H*-chromen-4-one (4j). A suspension of 2-(3,4-diaminophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (**4b**) (0.2 g, 0.64 mmol) and 4-(dimethylamino)butyric acid hydrochloride (3.2 g, 19.2 mmol) in 4 N HCl (10 mL) is heated at reflux for 48 h. After cooling, to the mixture sodium bicarbonate powder is carefully added until a basic condition is reached, and the solid precipitated is extracted with dichloromethane (3 × 20 mL). The organic phase is dried, filtered, and evaporated. The crude product is purified by flash chromatography (eluant dichloromethane/methanol in different ratios, 9:1, 8:2, 7:3, and 1:1) to yield **4j** as a yellowish solid (35%). ^{1}H NMR ($CDCl_3$) δ 2.00−2.10 (2H, m), 2.45 (6H, s), 2.60 (2H, t), 3.20 (2H, t), 4.00 (3H, s), 4.08 (3H, s), 6.80 (1H, s), 7.05 (1H, d), 7.60 (1H, d), 7.80 (1H, d), 7.95 (1H, d), 8.20 (1H, s). MS (ESI) *m/z* 408 [(M + H)$^+$]. Anal. ($C_{23}H_{25}N_3O_4$) C, H, N.

6-(7,8-Dimethoxy-4-oxo-4*H*-chromen-2-yl)-3-hydroxyquinoxalin-2(1*H*)-one (4k). To a solution of 2-(3,4-diaminophenyl)-7,8-dimethoxy-4*H*-chromen-4-one (4b) (0.1 g, 0.32 mmol) in anhydrous DMF (1 mL), at room temperature, 1,1′-oxalyldiimidazole (0.091 g, 0.48 mmol) is added and the mixture is stirred for 24 h. Methanol (5 mL) is added and the suspension is stirred at 50 °C for 30 min. After cooling, the yellow solid precipitated is filtered, washed with methanol and ether, and dried in a vacuum to obtain **4k** as a yellowish solid (51%). ^{1}H NMR (DMSO-d_6) δ 3.90−4.00 (6H, m), 6.80 (1H, s), 7.20−7.30 (2H, m), 7.70−7.85 (3H, m). MS (ESI) *m/z* 367 [(M + H)$^+$]. Anal. ($C_{19}H_{14}N_2O_6$) C, H, N.

2-(3,4-Diaminophenyl)-7,8-dihydroxy-4*H*-chromen-4-one (4c). A suspension of 2-(3,4-diaminophenyl)-7,8-dimethox

y-4*H*-chromen-4-one (**4b**) (0.1 g, 0.32 mmol) in aqueous hydrobromic acid (48%, 1 mL) is refluxed for 10 h. After cooling, the reaction mixture is diluted with water, neutralized with 20% $NaHCO_3$, and extracted with 1-butanol. The organic phase is washed with water, dried, evaporated under reduced pressure, and dried in a vacuum to obtain **4c** as a yellow solid (40%). ^{1}H NMR (DMSO-d_6) δ 5.60−6.00 (4H, m), 6.40 (1H, s), 6.65 (1H, d), 6.90 (1H, d), 7.30−7.40 (3H, m), 9.20 (1H, br s, ex), 10.20 (1H, br s, ex). MS (ESI) *m/z* 285 [(M + H)$^+$]. Anal. ($C_{15}H_{12}N_2O_4$) C, H, N.

By analogous procedures and starting from the appropriate chromen-4-ones, the following compounds were prepared:

2-(1*H*-Benzimidazol-5-yl)-7,8-dihydroxy-4*H*-chromen-4-one (4e). Yield 27%. ^{1}H NMR (DMSO-d_6) δ 6.88−6.92 (2H, m), 7.40 (1H, d), 7.80 (1H, d), 8.10 (1H, d), 8.50 (1H, s), 8.80 (2H, br s, ex). MS (ESI) *m/z* 295 [(M + H)$^+$]. Anal. ($C_{16}H_{10}N_2O_4$) C, H, N.

7,8-Dihydroxy-2-(2-hydroxy-1*H*-benzimidazol-5-yl)-4*H*-chromen-4-one (4g). Yield 60%. ^{1}H NMR (DMSO-d_6) δ 6.70 (1H, s), 6.95 (1H, d), 7.05 (1H, d), 7.38 (1H, d), 7.65 (1H, s), 7.75 (1H, d), 9.40 (1H, s), 10.25 (1H, s), 10.90 (1H, br s, ex), 10.95 (1H, br s, ex). MS (ESI) *m/z* 311 [(M + H)$^+$]. Anal. ($C_{16}H_{10}N_2O_5$) C, H, N.

7,8-Dihydroxy-2-(2-methyl-1*H*-benzimidazol-5-yl)-4*H*-chromen-4-one (4i). Yield 62%. ^{1}H NMR (DMSO-d_6) δ 2.55 (3H, s), 6.80 (1H, s), 6.95 (1H, d), 7.40 (1H, d), 7.60 (1H, d), 7.90 (1H, d), 8.30 (1H, s), 9.40 (1H, br s, ex), 10.20 (1H, br s, ex). MS (ESI) *m/z* 309 [(M + H)$^+$]. Anal. ($C_{17}H_{12}N_2O_4$) C, H, N.

6-(7,8-Dihydroxy-4-oxo-4*H*-chromen-2-yl)-3-hydroxyquinoxalin-2(1*H*)-one (4l). 54%. ^{1}H NMR (DMSO-d_6) δ 6.85 (1H, s), 7.26−7.38 (2H, m), 7.70−7.88 (3H, m), 9.20 (1H, br s, ex), 10.40 (1H, br s, ex). MS (ESI) *m/z* 339 [(M + H)$^+$]. Anal. ($C_{17}H_{10}N_2O_6$) C, H, N.

Flash-Plate Assay. To human telomerase buffer [HTB: 50 mM Tris−acetate, pH 8.5, 150 mM potassium acetate, 1 mM $MgCl_2$, and 1 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, EGTA] were added 10 μM dATP, 20 μM dGTP, 120 μM TTP (purchased from Amersham Biosciences), and 500 nM 5′-biotinylated primer (5′-bAATCCGTCGAGCAGAGTT). The reaction was started by adding to the above mixture telomerase enzyme obtained from cellular extracts of HEK293 cells overexpressing telomerase and enriched by affinity purification (kindly provided by Geron). The specific activity of the enzyme extract was adjusted to 5 fmol of dNTP incorporation min^{-1} μL^{-1} diluted in HTB containing 0.5 μM BSA.

The combined HTB, deoxynucleotides 5′-triphosphate, and the primers were aliquoted and distributed to individual wells of a 96-well microtiter plate. The test compounds, diluted in DMSO, were added individually to the wells at a dilution of 0.01−10 μM before the telomerase enzyme extract was added to start the reaction.

After 2 h of incubation at 37 ° C, the polymerization of telomeric repeats to the end of the primers was determined by hybridization in solution with 5 μg/mL ^{33}P-labeled (3′-dCCCTAACCCTAACCC), with a specific activity of 2500 Ci/mmol. This probe has been synthethized using a commercial kit (Amersham Biosciences), diluted in a buffer containing 1 M Tris-HCl, pH 7.0, 20× SSC (0.3 M sodium citrate, pH 7.0, containing 3 M NaCl), and 0.5 M ethylenediaminetetraacetic acid (EDTA). The hybridized oligonucleotides were then immobilized for quantification by transferring the reaction mixture to a streptavidin-coated Flash-Plate (purchased from NEN/Perkin-Elmer/Wallac Inc). After 2 h of incubation at 37 °C, the solution was removed and the wells were washed extensively with 2× SSC and 0.1% SDS. Finally, the amount of radiolabeled oligonucleotide was quantified by scintillation counting on a Packard TopCount.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Collins, K. Mammalian telomeres and telomerase. *Curr. Opin. Cell. Biol.* **2000**, *12*, 378−383.
(2) Harley, C. B. Telomere loss: mitotic clock or genetic time bomb? *Mutat. Res.* **1991**, *256*, 271−282.
(3) Shay, W. J.; Wright, W. E.; Werbin, H. Defining the molecular mechanisms of human cell immortalization. *Biochim. Biophys. Acta− Rev. Cancer* **1991**, *1072*, 1−7.
(4) Shay, W. J.; Bacchetti S. A Survey of Telomerase Activity in Human Cancer. *Eur. J. Cancer* **1997**, *33*, 787−791.
(5) Perry; P. J.; Jenkins, T. C. Recent advances in the development of telomerase inhibitors for the treatment of cancer. *Exp. Opin. Invest. Drugs* **1999**, *8* (12), 1981−2008.
(6) Neidle, S.; Kelland, L. R. Telomerase as an anti-cancer target: current status and future prospects. *Anti-Cancer Drug Des.* **1999**, *14*, 341−347.
(7) Ueno, T.; Takahashi, H.; Oda, M.; Mizunuma, M.; Yokoyama, A.; Goto, Y.; Mizushina, Y.; Sagakuchi, K.; Hayashi, H. Inhibition of Human Telomerase by Rubromycins: Implication of Spiroketal System of the Compounds as an Active Moiety. *Biochemistry* **2000**, *39*, 5995−6002.
(8) Ken-ichi, T.; Hideaki, K.; Masayuki, M.; You-Xin, S.; Hiroyuki, O. Inhibition of human telomerase activity by alterperylenol. *Oncol. Res.* **1998**, *10*, 449−453.
(9) Yuji, T.; Shuji, I.; Takashi, Y.; Toru, S.; Shigeru, H.; Sadatoshi, S.; Kazauo, S.; Haruo, S. Diazaphilonic acid a new azaphilone with telomerase inhibitory activity. *J. Antibiot.* **1999**, *52*, 412−414.
(10) Naasani, I.; Seimiya, H.; Tsuruo, T. Telomerase Inhibition, Telomere Shortening, and Senescence of Cancer Cells by Tea Catechins. *Biochem. Biophys. Res. Commun.* **1998**, *249*, 391−396.
(11) Sang Sun, K.; Seung Eu, L.; Growth and telomerase inhibition of SK-MEL 28 melanoma cell line by a plant flavonoid, apigenin. *J. Biochem. Mol. Biol.* **1998**, *31*, 339−344.
(12) Deschner, E.; Ruperto, J.; Wong, G.; Newmark, H. L. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* **1991**, *12* (7), 1193−1196.
(13) Elangovan, V.; Sekar, N.; Govindasamy, S. Chemopreventive potential of dietary bioflavonoids against 20-methylcholanthrene-induced tumorigenesis. *Cancer Lett.* **1994**, *87*, 107−113.

(14) Kuo, S. Antiproliferative potency of structurally distinct dietary flavonoids on human colon cancer cells. *Cancer Lett.* **1996**, *110*, 41−48.
(15) Chu, S. C.; Hsieh, Y. S.; Lin, J. Y. Inhibitory effects of flavonoids on moloney murine leukemia virus reverse transcriptase activity. *J. Nat. Prod.* **1992**, *55* (2), 179−183.
(16) Ono, K.; Nakane, H.; Fukushima, M.; Chermann, J. C.; Barrè-Sinoussi, F. Differential inhibitory effects of various flavonoids on the activities of reverse transcriptase and cellular DNA and RNA polymerases. *Eur. J. Biochem.* **1990**, *190*, 469−476.
(17) Badhwar, I. C.; Kang, K. S.; Venkataraman, K. Synthetical experiments in the chromone group. Part VII. Synthesis of 7,8,4′-trihydroxy-, 7,8,3′,4′-tetrahydroxy-, and 7,8,3′,4′,5′-, 5,7,3′,4′,5′-, and 3,7,3′,4′,5′-pentahydroxyflavones. *J. Chem. Soc.* **1932**, 1107−1112.
(18) Nagarathnam, D.; Cushman, M. A short and facile synthetic route to hydroxylated flavones. New syntheses of apigenin, tricin, and luteolin. *J. Org. Chem.* **1991**, *56* (16), 4884−4887.
(19) Klinke, P.; Gibian, H. Uber chalkone. *Chem. Ber.* **1961**, *94*, 26−38.
(20) Baker, W. Molecular rearrangement of some o-acyloxyacetophenones and the mechanism of the production of 3-acylchromones. *J. Chem. Soc.* **1933**, 1381−1388.
(21) Mahal, H. S.; Venkataraman, K. Synthetical experiments in the chromone group. Part XIV. The action of sodamide on 1-acyloxy-2-acetonaphthones. *J. Chem. Soc.* **1934**, 1767−1769.
(22) Wheeler, T. S. Flavone. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, pp 478−481.
(23) Kato, S.; Morie, T.; Yoshida, N. Synthesis and biological activity of 4-amino-5-chloro-2-ethoxy-3-hydroxybenzamides, metabolites of a new gastroprokinetic agent, mosapride. *Chem. Pharm. Bull.* **1996**, *44* (8), 1484−1492.
(24) Awad, W. I.; El-Neweihy, M. F.; Selim, S. F. Studies on 3-acylcathecols. II.1 A new synthesis of 8-hydroxyflavone. *J. Org. Chem.* **1960**, *25* (8), 1333−1336.
(25) Davis, F. A.; Han, W.; Murphy, C. K. Selective, electrophilic fluorinations using *N*-fluoro-*o*-benzenedisulfonimide. *J. Org. Chem.* **1995**, *60* (15), 4730−4737
(26) Pelter, A.; Ward, R. S.; Whalley, J. L. A facile synthesis of 2-substituted isoflavones for immunoassay: assembly of the isoflavonoid skeleton by means of a novel cyclisation reaction. *Synthesis* **1998**, *12*, 1793−1802.
(27) De Meyer, N.; Haemers, A.; Mishra, L.; Pandey, H. K.; Pieters, L. A. C.; Vanden Berghe, D. A.; Vlietinck, A. J. 4-Hydroxy-3-methoxyflavones with potent antipicornavirus activity. *J. Med. Chem.* **1991**, *34* (2), 736−746.
(28) Newman, M. S.; Ferrari, J. L.; Garg, C. P. A new synthesis of 3-chloroflavones. *J. Heterocycl. Chem.* **1964**, *1*, 76−78.
(29) Goker, H.; Ayhan, G.; Tuncbilek, M.; Ertan, R.; Leoncini, G.; Garzoglio, R.; Mazzei, M. Synthesis and antiaggregator activity of some new derivatives of 4*H*-1-benzopyran-4-one. *Eur. J. Med. Chem.* **1995**, *30*, 561−567.
(30) Bhatt, M. V.; Kulkarni, S. U. Cleavage of ethers. *Synthesis* **1983**, *4*, 249−282.
(31) Phillips, M. A. The formation of 2-methylbenzimidazoles. *J. Chem. Soc.* **1928**, 172−177.
(32) Goker, H.; Tuncbilek, M.; Ayhan, G.; Altanlar, N. Synthesis of some new benzimidazolecarboxamides and evaluation of their antimicrobial activity. *Farmaco* **1998**, *53* (6), 415−420.
(33) Nefzi, A.; Giulianotti, M. A.; Houghten, R. A. Solid-phase synthesis of branched thiohydantoin tetrahydroquinoxalinediones. *Tetrahedron Lett.* **2000**, *41* (14), 2283−2287.
(34) Asai, A.; Oshima, Y.; Yamamoto, Y.; Uochi, T.; Kusaka, H.; Akinaga, S.; Yamashita, Y.; Pongracz, K.; Pruzan, R.; Wunder, E.; Piatyszek, M.; Li, S.; Chin, A. C.; Harley, C. B.; Gryaznov, S. A Novel Telomerase Template Antagonist (GRN163) as a Potential Anticancer Agent. *Cancer Res.* **2003**, *63* (14), 3931−3939.
(35) Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L. C.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. Specific association of human telomerase activity with immortal cells and cancer. *Science* **1994**, *266*, 2011−2015.
(36) Mukai, S.; Kondo, Y.; Koga, S.; Komata, T.; Barna, B. P.; Kondo, S. 2−5A Antisense telomerase RNA therapy for intracranial malignant gliomas. *Cancer Res.* **2000**, *60* (16), 4461−4467.
(37) Kotic, S.; Soskic, V.; Joksimovic, J. Synthesis and evaluation of several cathechol bioisosteres as potential dopamine receptor ligands. *Bioorg. Med. Chem. Lett.* **1991**, *1* (8), 403−406.
(38) Arnett, C. D.; Wright, J.; Zenker, N. Synthesis and adrenergic activity of benzimidazole bioisosteres of norepinephrine and isoproterenol. *J. Med. Chem.* **1978**, *21*, 72−78.
(39) Perry, P. J.; Jenkins, T. C. DNA Tetraplex-binding drugs structure-selective targeting is critical for antitumor telomerase inhibition. *Mini Rev. Med. Chem.* **2001**, *1* (1), 31−41.
(40) Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins, T. C.; Kelland, R. L.; Neidle, S. 1,4- and 2,6-Disubstituted amidoanthracene-9,10-dione derivatives as inhibitors of human telomerase. *J. Med. Chem.* **1998**, *41*, 3253−3260.
(41) Caprio, V.; Guyen, B.; Opoku-Boahen, Y.; Mann, J.; Gowan, S. M.; Kelland, L. M.; Read, M. A.; Neidle, S. A novel inhibitor of human telomerase derived from 10*H*-indolo[3,2-*b*]quinoline. *Bioorg. Med. Chem. Lett.* **2000**, *10* (18), 2063−2066.

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