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Inhibition of Telomerase by BIBR 1532 and Related Analogues

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Abstract—BIBR 1532 has been reported to be a potent, small molecule inhibitor of human telomerase, suggesting it as a lead for the development of anti-telomerase therapy. We confirm the ability of BIBR 1532 to inhibit telomerase and report the discovery of an equally potent analogue. Importantly, IC_{50} values in cell extract are considerably higher than those previously reported using assays for purified enzyme, indicating that substantial improvement may be necessary.

Linear chromosomes are capped by telomeres containing variable numbers of nucleotide repeats, TTAGGG/AATCCC in humans.[1] Telomeres protect the gene encoding regions of chromosomes and are essential for maintaining the stability of the genome. Telomeres cannot be fully replicated by standard DNA polymerases and, in the absence of any mechanism for maintaining telomere length, telomeres will erode 50–100 bases per population doubling.[2]

In humans, a solution to the problem of maintaining telomere length is provided by telomerase, a ribonucleoprotein with one RNA (hTR) and one protein component (hTERT).[3–6] Telomerase has attracted great attention as a target for chemotherapy because telomerase activity is expressed in most tumor types but not in adjacent normal tissue.[7,8] This correlation has led to two related hypotheses: (1) reactivation of telomerase is necessary for maintaining viable telomeres during the rapid cell proliferation that characterizes cancer and (2) inhibition of telomerase may cause the telomeres of cancer cells to erode to a critical length and reduce the rate of tumor cell proliferation.

The hypothesis that telomerase is a novel target for cancer chemotherapy is supported by numerous observations of reduced cell growth upon telomerase inhibition by antisense RNA,[4] ribozymes,[9] expression of a dominant negative hTERT,[10,11] oligonucleotides,[12–14] or small molecules.[16–19] To date, the most potent inhibitors have been phosphoramidate or 2′-methoxyethyl oligonucleotides complementary to the telomerase RNA template.[12,13] While oligonucleotides are generating increasing excitement as potential drugs,[15] the advantages of small molecules continue to attract interest to their development as telomerase inhibitors. To date, several small molecule inhibitors of telomerase function have been developed,[16–20] many of which act to stabilize G-quadruplex structure.

One recently described small molecule, BIBR 1532, has been reported to be a promising telomerase inhibitor. It causes telomeres to shorten and reduces tumor cell proliferation.[18] Here, we describe the synthesis of BIBR 1532 and derivatives, and their ability to inhibit human telomerase.

Inhibitor Syntheses

Our strategy for the preparation of the inhibitors **1** is outlined in Figure 1 and utilized the high-yield addition of methyl anthranilate (**3**) to the acid chloride derived via standard procedures from **2** followed by aryl ester saponification. The key intermediate, **2**, was obtained by a novel Cr(II)-mediated condensation between commercial carbonyl **4** and either methyl 2,2-dichloro- or 2,2,2-trichloroacetates (**5**). The latter reaction[21] is notable for its excellent yields and unprecedented *Z*-stereoselectivity with trihaloacetates.[22,23]

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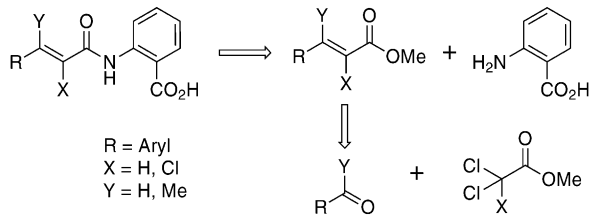

Figure 1. Retrosynthetic analysis.

Inhibition of Telomerase by BIBR 1532 and Derivatives

We assayed inhibition of telomerase using the telomeric repeat amplification protocol (TRAP).[24] TRAP is a sensitive assay that uses polymerase chain reaction (PCR) to amplify telomerase activity and we worked within the linear range of the assay. TRAP uses an internal amplification standard to insure that addition of inhibitor is not interfering with the amplification step. HeLa cells, derived from human cervical carcinoma, were lysed to obtain nuclear extract and the extract was used as a source for telomerase activity.

Using TRAP, we found that BIBR 1532 inhibited telomerase with an IC_{50} value of 5 μM. The *cis* isomer of BIBR 1532 (221-32) was also an inhibitor, with an IC_{50} value of 5 μM (Figs. 2 and 3). None of the other compounds tested inhibited telomerase at concentrations as high as 50 μM. The IC_{50} value of BIBR 1532 is substantially above the IC_{50} value, ~100 nM, reported by Damm and coworkers.[19,20]

One source of the different values is that our assays were conducted in a crude cell extract, while the previous work employed purified telomerase. The crude extract may contain interfering cellular components that reduce the potency of inhibitor, possibly by binding to it or modifying it. Alternatively, the crude extracts may contain a factor that interacts with telomerase and limits the ability of BIBR 1532 to be an inhibitor. Other workers have directly compared the IC_{50} values for BIBR 1532 in cell extract and with purified enzymes and report similar differences in inhibitory potency (Harley and Chin, personal communication).

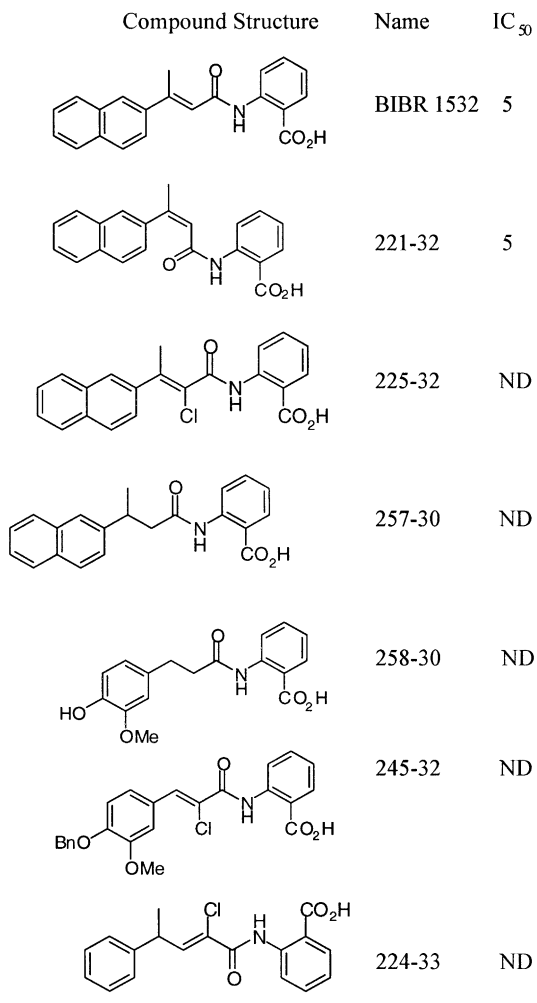

Figure 2. Compounds synthesized in these studies and the IC_{50} values that characterize their inhibition of telomerase. ND, inhibition not detected at concentrations as high as 50 μM.

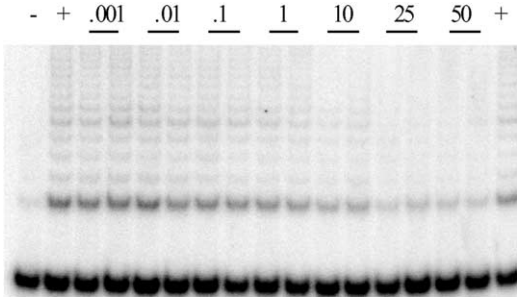

Figure 3. Inhibition of telomerase by BIBR 1532 monitored by TRAP. (−) Buffer only. (+) Cell extract only, no inhibitor added. Concentrations of BIBR 1532 ranged from 0.001 to 50 μM. Experiments were performed in duplicate. The lowest band is the internal amplification standard.

Conclusion

We have used a novel synthetic method to synthesize the anti-telomerase agent BIBR 1532 and related compounds. We find that both BIBR 1532 and the one active derivative (221-32) have IC_{50} values of 5 μM, substantially above the IC_{50} values for BIBR 1532 reported in the literature.[19] Our results emphasize the importance of considering the purity of enzyme when evaluating IC_{50} values and comparing values between laboratories, and suggest that the synthesis of derivatives of BIBR 1532 that are potent enough to use in clinical trials may be more challenging than had been anticipated.

Our data impact our working model for telomerase recognition of inhibitors by reinforcing the conclusion that it is difficult to identify highly potent small molecule lead compounds. Potent inhibition can be achieved by use of oligonucleotides.[12–14] Combined, these results suggest that in the near term oligonucleotides, not small molecules, may be better suited for use in clinical trials of anti-telomerase therapy.

Assay for Telomerase Activity

Telomerase activity was determined with the telomere repeat-amplification protocol (TRAP) and analyzed as described using whole cell lysate.[24]

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General procedure: preparation of (*Z*)-α-chloro-α,β-unsaturated esters and α,β-unsaturated esters

A solution of aldehyde or ketone (1 equiv) and methyl 2,2,2-trichloroacetate or 2,2-dichloroacetate (1.2 equiv) in dry THF (1 mL/mmol RCHO) was added to a stirring, room temperature suspension of commercial anhydrous $CrCl_2$ (5 equiv) in THF (10 mL/100 mg $CrCl_2$) under an argon atmosphere. Methyl 2,2,2-trichloroacetate reactions were continued at rt for 0.5 h whereas methyl 2,2-dichloroacetate reactions required 12 h at rt (or 2 h at 60 °C). The reddish, heterogenous reaction mixture was quenched with ice water and extracted thrice with Et_2O. The combined ethereal extracts were washed with brine, dried, and evaporated in vacuo. Chromatographic purification of the residue on SiO_2 afforded (*Z*)-α-chloro-α,β-unsaturated ester or α,β-unsaturated ester, respectively, in 98–100% yield.

General procedure: ester hydrolysis

LiOH (1 M aq solution, 4 equiv) was added to a room temperature solution of the above methyl ester in THF/H_2O (5:1, 10 mL/100 mg ester). After 12 h, the reaction mixture was acidified to pH 4 using 1 M aq oxalic acid solution, extracted thrice with EtOAc, and the combined organic extracts were evaporated in vacuo. Chromatographic purification of the residue on SiO_2 gave the corresponding carboxylic acid in 90–98% yield.

General procedure: amidation

A solution of carboxylic acid (1 equiv) in $SOCl_2$ (5 mL/100 mg acid) containing 1–2 drops (catalytic) of DMF was heated under reflux for 2 h. After cooling, all volatiles were removed under reduce pressure and the crude acid chloride was dissolved in ether (5 mL/100 mg acid) under an argon atmosphere. To this was added methyl anthranilate (1.1 equiv) followed by pyridine (1.3 equiv). After stirring at room temperature for 5 h, the reaction mixture was poured into 1 N HCl (5 mL/100 mg acid), extracted thrice with Et_2O, and the combined ethereal extracts were evaporated in vacuo. Chromatographic purification of the residue on SiO_2 furnished the corresponding methyl anthrailamide in 91–95% yield.

Methyl (*E*)- and (*Z*)-3-(naphthalen-2-yl)-but-2-enoates. Methyl 2,2-dichloroacetate and 2′-acetonaphthone were condensed in accordance with the general procedure to give methyl 3-(naphthalen-2-yl)-but-2-enoate as a chromatographically separable (SiO_2: 15% EtOAc/hexanes) *trans*/*cis*-mixture (70:30) whose spectral data were identical with the literature values.[25]

3-(Naphthalen-2-yl)-but-2(*E*)-enoic acid. All the spectral data were consistent with the literature values.[26]

***N*-(2-Carbomethoxyphenyl)-3-(naphthalen-2-yl)-but-2(*E*)-enamide**. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.76 (d, 3H, $J = 1.2$ Hz),

3.93 (s, 3H), 6.39 (d, 1H, J = 1.5 Hz), 7.04–7.12 (m, 1H), 7.45–7.66 (m, 4H), 7.80–7.89 (m, 3H), 7.97 (d, 1H, J = 1.81 Hz), 8.04 (dd, 1H, J = 1.5, 8.1 Hz), 8.88 (dd, 1H, J = 0.9, 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.12, 52.53, 114.94, 120.50, 121.35, 122.52, 124.28, 125.99, 126.65, 126.65, 126.76, 127.78, 128.32, 128.67, 131.08, 133.35, 133.59, 134.86, 139.92, 142,22, 153.43, 165.62, 169.06. MS: m/z 345 (M^+).

***N*-(2-Carboxyphenyl)-3-(naphthalen-2-yl)-but-2(*E*)-enamide (BIBR 1532)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.75 (d, 3H, J = 1.2 Hz), 6.43 (s, 1H), 7.17 (t, 1H, J = 8.4 Hz), 7.40–7.80 (m, 4H), 7.81–7.84 (m, 3H), 8.15–8.20 (m, 2H), 8.88 (dd, 1H, J = 0.90, 8.40 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.16, 109.68, 120.97, 123.68, 127.13, 127.20, 127.78, 127.89, 128.89, 129.07, 129.73, 133.16, 134.38, 135.30, 137.99, 140.99, 151.89, 162.22, 165.25. MS: m/z 331 (M^+).

***N*-(2-Carbomethoxyphenyl)-3-(naphthalen-2-yl)-but-2(*Z*)-enamide**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.76 (d, 3H, J = 0.9 Hz), 3.93 (s, 3H), 6.39 (d, 1H, J = 1.2 Hz), 7.09 (t, 1H, J = 8.1 Hz), 7.40–7.68 (m, 4H), 7.80–8.05 (m, 5H), 8.70 (d, 1H, J = 8.40 Hz), 11.26 (s, 1H). MS: m/z 345 (M^+).

***N*-(2-Carboxyphenyl)-3-(naphthalen-2-yl)-but-2(*E*)-enamide (221-32)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.70 (d, 3H, J = 1.2 Hz), 6.44 (d, 1H, J = 1.50 Hz), 7.08–7.15 (m, 1H), 7.40–7.61 (m, 3H), 7.72 (dd, 1H, J = 1.8, 8.7 Hz), 7.82–8.00 (m, 3H), 8.10–8.17 (m, 2H), 8.85 (dd, 1H, J = 0.9, 8.1 Hz), 11.45 (s, 1H). MS: m/z 331 (M^+).

Methyl 2-chloro-3-(naphthalen-2-yl)-but-2(*Z*)-enoate. Methyl 2,2,2-trichloroacetate and 2′-acetonaphthone were condensed in accordance with the general procedure. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.37 (s, 3H,–CH_3), 3.46 (s, 3H, –CO_2Me), 7.25–7.30 (m, 1H), 7.42–7.52 (m, 2H), 7.62 (s, 1H), 7.76–7.84 (m, 3H). MS: m/z 260 (M^+), 262 ($M^+ + 2$).

***N*-(2-Carbomethoxyphenyl)-2-chloro-3-(naphthalen-2-yl)-but-2(*Z*)-enamide.** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.43 (s, 3H), 3.65 (s, 3H), 6.94–7.02 (m, 1H), 7.20–7.45 (m, 4H), 7.67–7.85 (m, 5H), 8.54 (d, 1H, J = 8.4 Hz), 11.27 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.69, 52.27, 115.56, 120.67, 123.12, 124.23, 125.69, 126.41, 126.45, 126.62, 127.80, 128.06, 128.32, 130.81, 132.94, 133.29, 134.55, 137.80, 140.71, 142.46, 162.82, 168.07. MS: m/z 379 (M^+), 381 ($M^+ + 2$).

***N*-(2-Carboxyphenyl)-2-chloro-3-(naphthalen-2-yl)-but-2(*Z*)-enamide (225-32)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.45 (s, 3H), 7.09 (t, 1H, J = 6.9 Hz), 7.32–7.45 (m, 3H), 7.52 (t, 1H, J = 9.0 Hz), 7.70–7.80 (m, 4H), 8.01 (dd, 1H, J = 1.2, 7.80 Hz), 8.60 (d, 1H, J = 8.7 Hz), 11.05 (s, 1H). MS: m/z 365 (M^+).

Methyl 2-chloro-4-phenyl-pent-2(*Z*)-enoate. Methyl 2,2,2-trichloroacetate and 2-phenylpropionaldehyde were condensed in accordance with the general procedure. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (d, 3H, J = 6.6 Hz, CH_3), 3.81 (s, 3H, CO_2CH_3), 4.35–4.15 (m, 1H), 7.60 (d, 1H, J = 9.6 Hz), 7.20–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.10, 39.75, 53.29, 123.24, 127.13, 127.26, 128.98, 142.92, 146.15, 163.28. MS: m/z 224 (M^+), 226 ($M^+ + 2$).

***N*-(2-Carbomethoxyphenyl)-2-chloro-4-(phenyl)-pent-2(*Z*)-enamide**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47 (d, 3H, J = 7.2 Hz), 3.93 (s, 3H), 4.05–4.17 (m, 1H), 7.06–7.14 (m, 1H), 7.20–7.40 (m, 6H), 7.51–7.60 (m, 1H), 8.03 (dd, 1H, J = 1.5, 7.8 Hz), 8.75 (dd, 1H, J = 0.9, 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.54, 39.84, 52.63, 116.13, 120.72, 123.37, 125.28, 126.95, 127.27, 128.88, 131.12, 134.68, 140.86, 143.23, 143.32, 163.63, 168.47. MS: m/z 343 (M^+), 345 ($M^+ + 2$).

***N*-(2-Carboxyphenyl)-2-chloro-4-(phenyl)-pent-2(*Z*)-enamide (224-33)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.70 (d, 3H, J = 1.2 Hz), 4.00–4.20 (m, 1H), 7.10–7.36 (m, 6H), 7.40 (d, 1H, J = 8.7 Hz), 7.47–7.60 (m, 1H), 8.02 (dd, 1H, J = 1.5, 7.8 Hz), 8.76 (dd, 1H, J = 0.9, 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.63, 40.01, 115.23, 120.92, 123.72, 125.11, 127.05, 127.29, 128.95, 132.19, 135.87, 141.37, 143.16, 143.91, 160.85, 173.09. MS: m/z 329 (M^+).

***N*-(2-Carboxyphenyl)-3-(naphthalen-2-yl)-butenamide (257-30).** *N*-(2-Carboxyphenyl)-2-chloro-3-(naphthalen-2-yl)-but-2(*Z*)-enamide was stirred with 10% Pd/C in MeOH under a hydrogen atmosphere (1 atm) for 3 h. The reaction mixture was filtered and all volatiles remove in vacuo to give the title compound in 98% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (d, 3H, J = 6.9 Hz), 2.72 (dd, 1H, J = 7.8, 14.4 Hz), 2.85 (dd, 2H, J = 6.9, 14.4 Hz), 3.54–3.70 (m, 1H), 7.04 (t, 1H, J = 7.2 Hz), 7.30–7.42 (m, 3H), 7.45–7.81 (m, 1H), 7.62–7.80 (m, 4H), 8.05 (d, 1H, J = 8.10 Hz), 8.75 (d, 1H, J = 8.2 Hz), 11.04 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.78, 37.30, 47.61, 114.68, 120.88, 123.13, 125.16, 125.57, 125.61, 126.14, 127.71, 127.81, 128.44, 131.88, 132.50, 133.73, 135.57, 141.69, 143.07, 171.76, 172.27. MS: m/z 333 (M^+).

Methyl 2-chloro-3-(3-methoxy-4-benzyloxyphenyl)-prop-2(*Z*)-enoate. Methyl 2,2,2-trichloroacetate and 3-methoxy-4-benzyloxybenzaldehyde were condensed in accordance with the general procedure. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.89 (s, 3H,–CO_2CH_3), 3.93 (s, 3H, –OCH_3), 5.21 (s, 2H), 6.91 (d, 1H, J = 8.4 Hz), 7.28–7.40 (m, 4H), 7.43 (d, 2H, J = 7.2 Hz), 7.57 (s, 1H), 7.84(s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 53.46, 56.19, 70.92, 113.16, 113.73, 119.47, 125.42, 126.23, 127.37, 128.23, 128.84, 136.64, 137.15, 149.33, 150.23, 164.35. MS: m/z 332 (M^+), 334 ($M^+ + 2$).

***N*-(2-Carbomethoxyphenyl)-2-chloro-3-(3-methoxy-4-benzyloxyphenyl)-prop-2(*Z*)-enamide (245-32)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.93 (s, 3H), 3.97 (s, 3H), 5.22 (s, 2H), 6.92 (d, 1H, J = 8.4 Hz), 7.09–7.20 (m, 1H), 7.30–7.50 (m, 6H), 7.52–7.64 (m, 2H), 8.03 (s, 1H), 8.08 (dd, 1H, J = 1.5, 7.8 Hz), 8.83 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 52.73, 56.19, 70.97, 113.28, 113.69, 116.26, 120.85, 121.83, 123.41, 125.12, 126.64, 127.40, 128.23, 128.26, 131.23, 134.75, 134.79, 136.78, 141.13, 149.33, 149.91, 161.73. MS: m/z 451 (M^+), 453 ($M^+ + 2$).

***N*-(2-Carboxyphenyl)-2-chloro-3-(3-methoxy-4-benzyloxyphenyl)-prop-2(*Z*)-enamide (258-30)**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 3H), 5.18 (s, 2H) 7.05–7.72 (m, 10H), 8.00–8.10 (m, 2H), 8.71 (d, 1H, J = 8.7 Hz). MS: m/z 437 (M^+).

***N*-(2-Carboxyphenyl)-3-(3-methoxy-4-hydroxyphenyl)-propanamide.** *N*-(2-Carboxyphenyl)-2-chloro-3-(3-methoxy-4-benzyloxyphenyl)-prop-2(*Z*)-enamide was stirred with 10% Pd/C in MeOH under a hydrogen atmosphere (1 atm) for 3 h. The reaction mixture was filtered and all volatiles remove in vacuo to give the title compound in 98% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.68 (t, 2H, J = 8.1 Hz), 2.93 (t, 2H, J = 7.5 Hz), 3.78 (s, 3H), 6.71 (d, 2H, J = 1.2 Hz), 6.90 (s, 1H), 7.09 (t, 1H, J = 8.1 Hz), 7.53 (t, 1H, J = 8.1 Hz), 8.10 (dd, 1H, J = 1.5, 7.8 Hz), 8.73 (dd, 1H, J = 1.5, 7.8 Hz), 11.60 (s, 1H). MS: m/z 315 (M^+).