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LETTER ARTICLE

Inhibition of *Taq* DNA Polymerase and Human Topoisomerase I by Resveratrol Derivatives

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ARTICLE HISTORY

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Abstract: ***Background:*** DNA polymerases and topoisomerases are proteins that play a vital part in DNA metabolism such as replication, transcription, recombination, and chromosome segregation during mitosis. For this reason, these enzymes are appreciated targets for the development of cancer chemotherapeutic drugs.

Methods: All compounds evaluated in this work were obtained under the conditions and using the reagents described in the Schemes (**1-3**). *Polymerase Chain Reaction (PCR):* Sample stock solutions were prepared at a concentration of 10 mM in dimethyl sulfoxide (DMSO) using 1 ml as a final volume. The PCR master mix consisted of 2.5 µl of PCR Buffer (40 mM Tris-acetate, pH 8.3), 0.5 µl of $MgCl_2$ at a concentration of 15 mM, 2.5 U *Taq* DNA polymerase, 2.5 µl of each oligonucleotide primer at a concentration of 100 mM, 0.5 µl of each deoxynucleotide triphosphates (dNTP) at a concentration of 10 mM and 0.1 µg/ml of DNA template**.** Topoisomerase I activity was determined by relaxation of supercoiled pBr 322 plasmid DNA. The reaction mixture in 20 ml contained 35 mM Tris-HCl, pH 8, 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 0.01% bovine serum albumin, 0.64 mg/20 ml supercoiled pBr 322 DNA, 1.5 U human topoisomerase I and 1 ml of test compound diluted with DMSO.

Results: Faithful replication of DNA molecules with the intervention of DNA polymerases and topoisomerases is essential for genome integrity and correct transmission of genetic information in all living organisms. For this reason, DNA polymerases and topoisomerases have emerged as important cellular targets for chemical intervention in the development of anti-cancer agents. Herein we report the semi-synthesis of resveratrol derivatives and similar compounds and their biological activity against *Taq* DNA polymerase and topoisomerases. Compound **4** was the most active against both enzymes with IC_{50} values equal to 18.56 and 28.37 µM, respectively. Additionally, compounds **3** and **5** showed interesting activity against *Taq* DNA polymerase with IC_{50} values equal to 76.89 and 71.65 µM, respectively.

Conclusion: In summary, one compound (**4**) was found to have excellent inhibitory activity against human topoisomerase I and *Taq* DNA polymerase. This compound can be a leading molecule for the development of original anticancer treatment and an excellent instrument to explore DNA polymerase and topoisomerase activity.

Keywords: Resveratrol, DNA polymerases, natural products, human topoisomerase I, stilbene.

1. INTRODUCTION

DNA polymerases and topoisomerases are proteins that play a vital role in DNA metabolism such as replication, transcription, recombination, and chromosome segregation during mitosis [1-3]. For this reason, these enzymes are appreciated targets for the development of cancer chemotherapeutic drugs. DNA topoisomerases, which catalyze the interconversion of numerous topological states of DNA, were initially revealed as enzymes that change the super helical structure of closed circular DNA. DNA topoisomerases can be classified into two categories according to their functional devices. Category I DNA topoisomerases break and rejoin only one of the two strands during catalysis, while type II DNA topoisomerases break and rejoin both strands for each DNA strand-passing reaction. Topoisomerase I seems to be associated with actively transcribed genes, whereas topoisomerase II is required for DNA replication and for successful traverse of mitosis [4]. Through these two different mechanisms, DNA topoisomerases change the topological states of DNA, which facilitate various DNA transactions such as DNA replication, RNA transcription, recombination, chromosome condensation/decondensation, and chromosome segregation [1-3]. Numerous inhibitors have been used in clinical trials including dideoxynucleotides, phospholipids, fatty acids, flavonoids, iridoids, triterpenoids, camptothecines, anthacyclines, aminoacridines and ellipticines [4, 5]. However, more active compounds are still necessary.

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DNA polymerases from different organisms share common features: all of them have three different activities located on different domains. Bacterial DNA polymerase has multidomain structures, which contain a 5'-3' exonuclease domain, a 3'-5' exonuclease domain and a polymerase domain. According to Astatke *et al.* [2], the last domain has a structure reminiscent of a right hand, with a large cleft formed by the fingers, thumb and palm domains. Comparison of the multidomains of DNA polymerases from dissimilar organisms shows that the hand-shape design is a common piece of the polymerase domain among all known DNA polymerases. There is substantial evidence showing that the area covering the junction between the palm and fingers subdomains has a big relevance in the binding and arriving of nucleotides. The prominence of these domains is also exposed by amino acid sequence conservation [1, 3].

Phenylpropanoids are an important group of the natural products. Stilbenes are a small group of phenylpropanoids characterized by a skeleton of 1,2-diphenylethylene. Most stilbene derivatives are polyphenolic compounds isolated from plant; some common examples of these compounds have been isolated from various families, including grape (Vitaceae), pineapple (Pinaceae), peanuts (Fabaceae) and sorghum (Poaceae) [6].

Resveratrol (3,5,4´-trihydroxystilbene) (**1**) is a polyphenolic compound from the stilbene family. The essential structure of this molecule comprises two aromatic rings linked by a methylene bridge. Resveratrol exists as two isoforms: *trans*-resveratrol y *cis*-resveratrol. The isomer *trans*- is the more stable form; the isomerization of the form *trans*- to form *cis*- is possible in the presence of UV light and conditions of elevated pH [7].

It has been shown that resveratrol arrests the cell cycle and induces apoptosis by inhibiting the main survival translation path signal. This compound inhibits the pathways of the activated protein kinase for mitogen and phosphatidylinositol 3-kinase (PI3K) / AKT. Consequently, it suppresses the activity of the transcription factors AP-1 "downstream" and nuclear factor kB (NF-kB) (Rel A / p65) [8].

In addition, resveratrol can give access to reactive molecules such as Cu (II), which, together with resveratrol could produce the strand cleavage and DNA "nicking". Also, it could affect the process involved in the metabolism of DNA by blocking the enzyme sites that interact with DNA, resulting in the inhibition of topoisomerases and catalytic polymerases, including telomerase [9].

Herein we report the semi-synthesis of resveratrol derivatives and similar compounds [10] and the inhibitory activity against *Taq* DNA polymerase and human Topoisomerase I [11] in order to evaluate the interaction of this kind of compound with the enzymes in a direct form.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The semi-synthesis of the compounds reported in this study is shown in Schemes (**1**-**3**). Compounds **3** and **4** were generated by benzoylation reactions of resveratrol, while compound **5** was obtained by benzylation reaction. Compound **2** was converted into compound **6** by epoxidation reaction. Then, compounds **13**-**18** and **22**-**23** were obtained from compound **11** by esterification reaction using dicyclohexylcarbodiimide (DCC) and finally compound **11** was converted into compounds **19**-**21** by silylation reactions.

2.2. Bioactivity

A group of 24 compounds at a concentration of 500 μM was assayed against *Taq* DNA polymerase and human topoisomerase I (Table **1**). To determine IC_{50} values, serial dilutions (1:2) were performed. Compound **4** was the most active against both enzymes with IC_{50} values equal to 18.56 and 28.37 μM, respectively. Additionally, compounds **3** and **5** showed interesting activity against *Taq* DNA polymerase with IC_{50} values equal to 76.89 and 71.65 μM, respectively.

2.3. Discussion

In the past years, progress in molecular biology has contributed to increasing understanding of the fundamental mechanisms associated to cancer origination, promotion and progression. Consequently, new anticancer compounds such as monoclonal antibodies and other compounds have been developed. These compounds affect specific molecular targets (normally proteins) involved in tumour progress and evolution, and consequently have become a significant portion of the anticancer armamentarium. These targets include growth factor receptors, signalling molecules, cell-cycle proteins, apoptosis modulators, and chemicals related with invasion and angiogenesis [5].

Some of these molecular targets are DNA polymerases, and topoisomerases that have lately appeared as significant cellular targets for chemical intervention in the development of anti-cancer agents because a correct replication of DNA chain by DNA polymerases is indispensable for genome integrity and precise transmission of genetic data in all living organisms.

The idea behind molecular targeting is to project actions that specifically attack the molecular ways that originate disease, without altering the usual functions in our cells. Medications developed using this approach can be less toxic and more operative than existing drugs.

Over the past years, there has been a strong debate about the molecular target implicated in the cytotoxic activity shown by resveratrol (**1**). From our results, it is possible to observe that although resveratrol has previously shown antiproliferative activity, human topoisomerase I and *Taq* DNA polymerase may not be the molecular target implicated in this activity. These results are in coincidence with those obtained by Wiesmüller's group [9], but they are in disagreement with observations by Webb *et al* [10]. This evidence could indicate that resveratrol (**1**) may decrease or inhibit the levels of topoisomerases and telomerases in the cells. For example, in the case of telomerases, resveratrol (**1**) decreases the catalytic activity of this enzyme by the inhibition of kinases that are important for the cellular transit of the hTert subunit of telomerases [9].

On the other hand, compound (**4**) was the only able to inhibit the catalytic activity of human topoisomerase I and

Table 1. Inhibitory activity at a concentration of 500 μM and IC_{50} values for the compounds panel.

Compound	*Taq* DNA Polymerase IC_{50} (μM)	Human Topoisomerase I IC_{50} (μM)	Compound	*Taq* DNA Polymerase IC_{50} (μM)	Human Topoisomerase I IC_{50} (μM)
1	140.22	NI	**13**	172.27	NI
2	160.42	NI	**14**	158.20	NI
3	76.89	NI	**15**	NI	NI
4	18.56	28.37	**16**	144.28	NI
5	71.65	NI	**17**	109.34	NI
6	170.84	NI	**18**	113.90	NI
7	NI	NI	**19**	131.43	NI
8	190.11	NI	**20**	105.16	NI
9	165.60	NI	**21**	97.29	NI
10	158.26	NI	**22**	99.06	NI
11	NI	NI	**23**	113.37	NI
12	NI	NI	**24**	137.76	NI
Inhibition Controls					
ddNTP set	0.13	-	**Camptothecin**	-	0.09

IC_{50} values were determinated by interpolation from plots of enzyme activity *vs*. inhibitor concentration. The IC_{50} values are means from at least three independent experiments and standart deviation never exceeded 20%. NI: No Inhibition. NT: No Tested. Comp.: compound. The results are expressed in μM.

also *Taq* DNA polymerase. Additionally, a large group of compounds showed inhibitory activity against *Taq* DNA polymerase. Considering this result, it is possible to suggest that these compounds are not acting directly on DNA as intercalators. If that had been the case, they would have inhibited both enzymes due to conformational changes that occur in the DNA structure. Therefore, the inhibitory activity for this group of compounds is probably generated by direct interaction with the structure of *Taq* DNA polymerase. This activity at molecular level may also explain the antiproliferative activity shown by some of these compounds [11, 12]. However, more specific assays will be necessary to elucidate the mechanism of action.

Additionally, it is interesting to mention that most active compounds (**3**-**5**) against both enzymes are derivatives from resveratrol (1). This phenomenon shows the relevance of resveratrol and derivatives as leading molecules in on the search of bioactive compounds for cancer.

3. MATERIALS AND METHODS

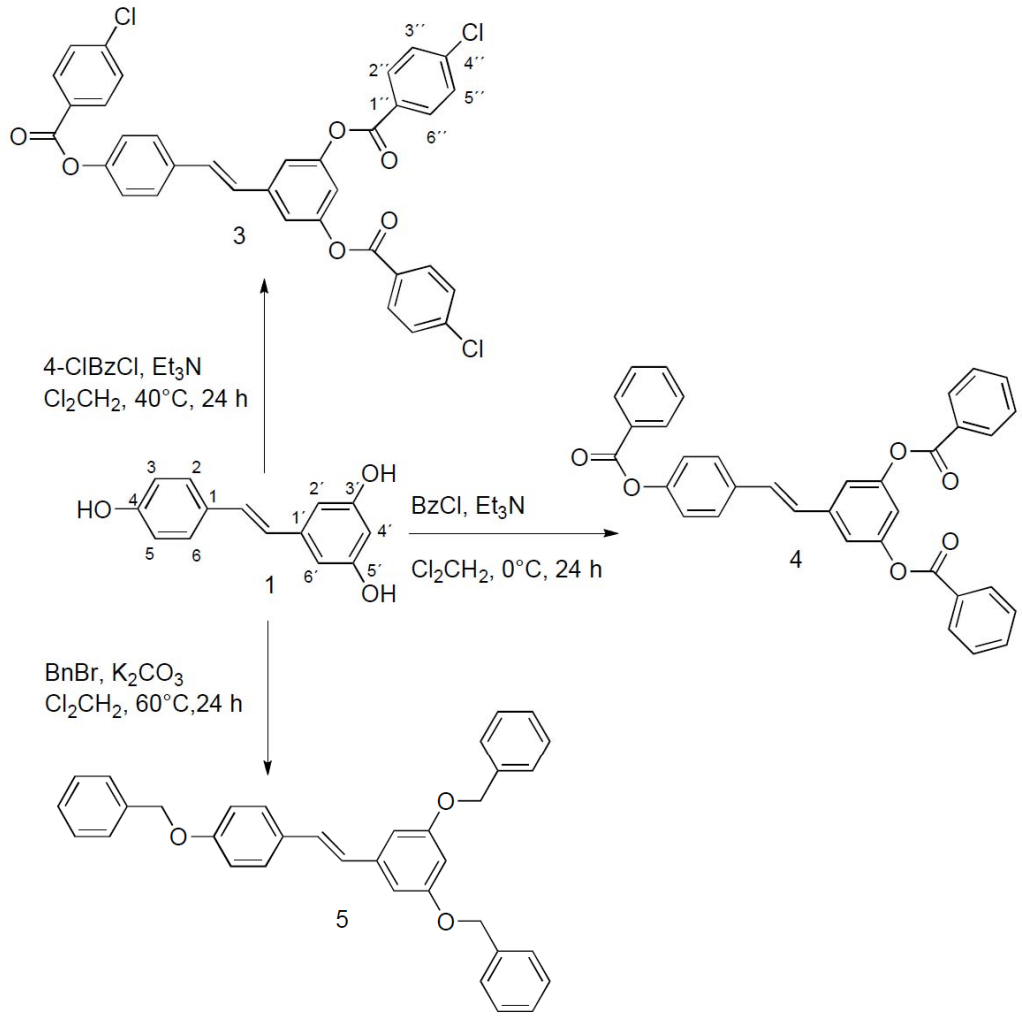
3.1. Chemical Methods

3.1.1. Chemicals and Reagents

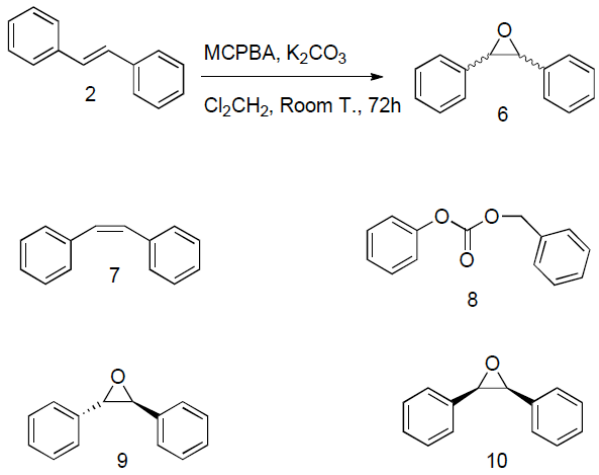
Commercial reagents for the preparation of compounds were obtained from Fluka, Sigma-Aldrich and Merck. Reactions requiring anhydrous conditions were performed under nitrogen or argon. Dichloromethane and diethyl ether were distilled from CaH_2 and Na°/benzophenone, respectively, under N_2 prior to use. Other solvents or chemicals were purified by standard techniques. Thin-layer chromatography was carried out on Merck and Macherey-Nagel aluminium sheets coated with silica gel 60 F_{254}. Plates were visualized by UV light and/or phosphomolybdic acid 20 wt. % solution in ethanol with heating. Anhydrous magnesium sulphate was used for drying solutions. Chromatography in column was performed on silica gel Merck grade 9385, 60 Å. *Compounds*: All compounds evaluated in this work were obtained under the conditions and using the reagents described in the Schemes (**1**-**3**).

3.1.2. Spectroscopic Data

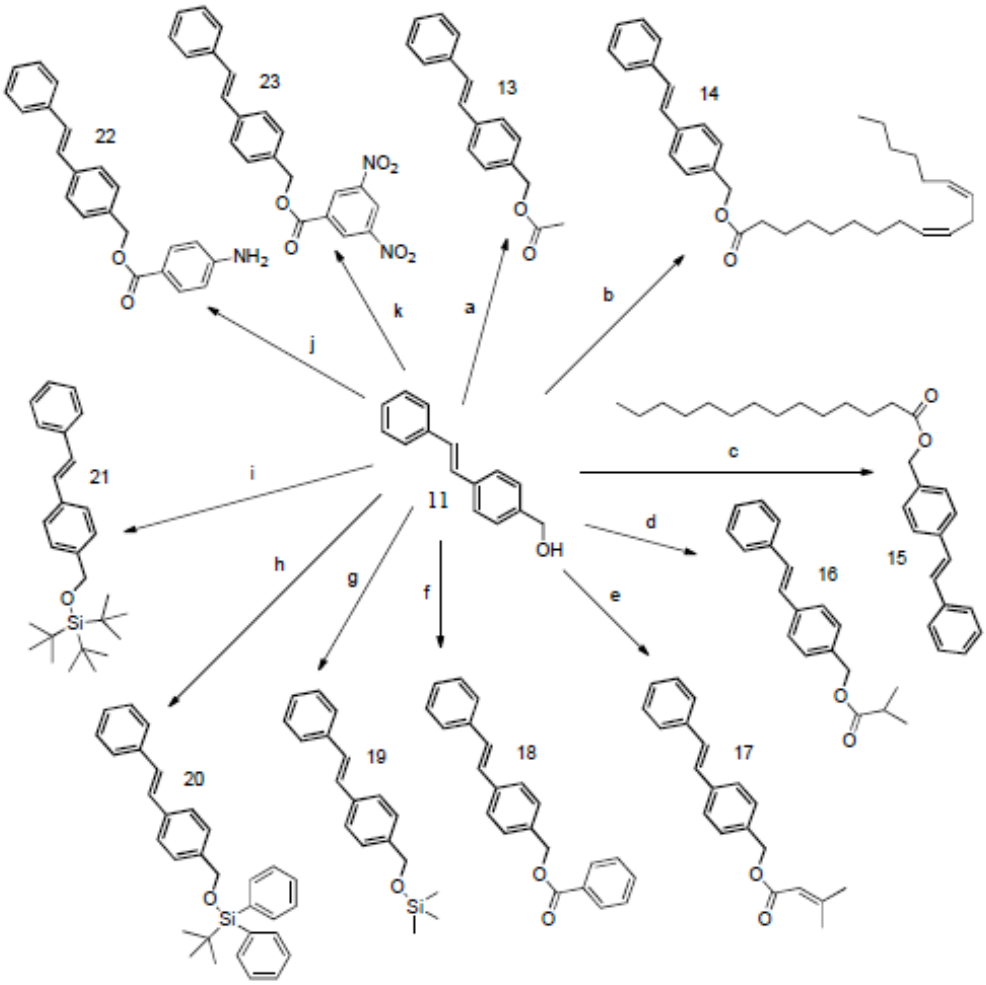
NMR spectra were measured at 200 and 400 MHz (^{1}H NMR) and 50 and 100 MHz (^{13}C NMR) with a Bruker AC-200 and 400 instruments, and chemical shifts are reported relative to internal Me_4Si (δ= 0). All intermediates and final products gave satisfactory analytical and spectroscopic data in full accordance with their assigned structures. Structural characterization by NMR of key compounds: Compound **3**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (6H, $H_{2''}$ and $H_{6''}$), 7.93 (2H, H_2 and H_6), 7.65 (6H, $H_{3''}$ and $H_{5''}$), 7.36 (2H, H_3 and H_5), 7.34 (2H, $H_{2'}$ and $H_{6'}$), 7.32 (1H, $H_{4'}$), 6.94 (2H, -CH=CH-). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.30 (C=O), 151.60 ($C_{3'}$ and $C_{5'}$), 148.70 (C_4), 139.50 ($C_{4''}$), 139.20 ($C_{1'}$), 134.20 (C_1), 131.80 ($C_{2''}$ and $C_{6''}$), 129.70 (C_2 and C_6), 128.60 ($C_{3''}$ and $C_{5''}$), 128.30 ($C_{1''}$), 127.40 (-CH=CH-), 121.50 (C_3 and C_5), 118.00 ($C_{2'}$ and $C_{6'}$), 115.30 ($C_{4'}$). Compound **4**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.21 (6H, $H_{2''}$ and



Scheme (1). Structures of reveratrol (**1**) and derivatives.



Scheme (2). Stilbenes and related compounds.



a. Acetic anhydride, pyridine, r.t., 24 hs. b. Linoleic acid, 4-DMAP, DCC, CH_2Cl_2, r.t, 24 hs. c. Mlristic acid, 4-DMAP, DCC, CH_2Cl_2, r.t., 24 hs. d. Isobutiric acid, 4-DMAP, DCC, CH_2Cl_2, r.t., 24 hs. e. 3,3-dimethylacrylic acid, 4-DMAP, DCC, CH_2Cl_2, r.t., 24 hs. f. Benzoic acid, 4-DMAP, DCC, CH_2Cl_2, r.t., 24 hs. g. TMSCl, imidazole, CH_2Cl_2, r.t., 24 hs. h. TBDPSCl, imidazole, CH_2Cl_2, r.t., 24 hs. i. TTBSCl, imidazole, CH_2Cl_2, r.t., 24 hs. j. p-amine-benzoic acid, 4-DMAP, DCC, CH_2Cl_2, r.t., 24 hs. k. 3,5-dinitro-benzoic acid, 4-DMAP, DCC, CH_2Cl_2, r.t., 24 hs.

Scheme (3). Trans-4-(methanol) stilbene (**11**), derivatives and others compounds.

$H_{6''}$), 7.93 (2H, H_2 and H_6), 7.72 (3H, $H_{4''}$), 7.61 (6H, $H_{3''}$ and $H_{5''}$), 7.36 (2H, H_3 and H_5), 7.34 (2H, $H_{2'}$ and $H_{6'}$), 7.30 (1H, $H_{4'}$), 6.98 (2H, -CH=CH-).^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.30 (C=O), 151.70 ($C_{3'}$ and $C_{5'}$), 148.50 (C_4), 139.40 ($C_{1'}$), 134.40 (C_1), 133.80 ($C_{4''}$), 130.20 ($C_{1''}$, $C_{2''}$ and $C_{6''}$), 128.70 ($C_{3''}$ and $C_{5''}$), 127.50 (-CH=CH-), 121.40 (C_3 and C_5), 118.10 ($C_{2'}$ and $C_{6'}$), 115.50 ($C_{4'}$). Compound **5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (2H, H_2 and H_6), 7.46 (6H, $H_{2''}$ and $H_{6''}$), 7.39 (9H, $H_{3''}$, $H_{4''}$ and $H_{5''}$), 6.98 (6H, H_3 and H_5), 6.94 (2H, -CH=CH-), 6.86 (2H, $H_{2'}$ and $H_{6'}$), 6.21 (1H, $H_{4'}$), 5.15 (6H, -OCH_2-) ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159,80 ($C_{3'}$ and $C_{5'}$), 158.10 (C_4), 136.60 ($C_{1'}$),130.30 ($C_{2'}$ and $C_{6'}$), 129.70 (C_1), 128.80 ($C_{3''}$ and $C_{5''}$), 127.50 (-CH=CH-), 127,20 ($C_{2''}$ and $C_{6''}$), 114.10 (C_3 and C_5), 105.60 ($C_{2'}$ and $C_{6'}$), 99.50 ($C_{4'}$), 70.70 (-CH_2).

3.2. Bioactivity

3.2.1. PCR

The ddNTPs, *Taq* recombinant *Thermus aquaticus* expressed in *Escherichia coli* D1806 and water MQ (Millie Q) were obtained from Sigma-Aldrich. *Polymerase Chain Reaction (PCR)*: Sample stock solutions were prepared at a concentration of 10 mM in dimethyl sulfoxide (DMSO) using 1 ml as final volume. The PCR master mix consisted of 2.5 µl of PCR Buffer (40 mM Tris-acetate, pH 8.3), 0.5 µl of

$MgCl_2$ at a concentration of 15 mM, 2.5 U *Taq* DNA polymerase, 2.5 µl of each oligonucleotide primer at a concentration of 100 mM, 0.5 µl of each deoxynucleotide triphosphates (dNTP) at a concentration of 10 mM and 0.1 µg/ml of DNA template (16S rRNA gene from genomic DNA of the Gram negative *Pseudomonas sp*.). In the experiments the primers were: sense 5′ AGAGTTTGATCMTGGCTCAG and antisense: 5′ GGTTACCTTGTTACGACTT. One microliter of compound solution at a concentration of 10 mM was added to the PCR master mix. The amplification conditions consisted of 30 cycles of denaturation at 95ºC for 60 s followed by primers annealing at 55ºC for 60 s and primer extension at 72ºC for 180 sec. Inhibition positive controls were performed using ddNTP set at a final concentration of 500 µM. Activity controls were executed without compounds solution in the master mix and solvent controls were performed without compounds but with 1µl of DMSO solvent. After completion of reaction 2 µl of loading buffer 10x (0.25% of bromophenol blue, 0.25% xylene cyanol FF, 15% Ficoll 400 in water) was added. The amplified DNA sequences were electrophoresed for 60 m in 0.8% agarose gel in buffer TBE 1x (Tris-boric-EDTA, pH: 8) at 75-80 V using TBE running buffer 1x. Finally, gels were stained using 0.5 µg of ethidium bromide per ml. Amplified DNA bands were detected visually with UV transilluminator. Each assay was replicated three times.

3.2.2. Analysis of PCR Products

The image of stained agarose gels was captured using a Photodocumentary UVP Imaging System. The digitized band images were processed using the Image processing program (Scion Image, public domain program).

3.2.3. IC_{50} Values Determination

Serial dilutions (1:2) were performed in DMSO (concentration 500 µM to 7.8125 µM). The PCR conditions (master mix, program cycles, electrophoresis and staining of gels) were the same that in the screening assay. IC_{50} values were determined using Scion Image (public domain) and GraphPad Prism 4 (demo version).

3.2.4. Human Topoisomerase I Assay

Topoisomerase I activity was determined by relaxation of supercoiled pBr 322 plasmid DNA. The reaction mixture in 20 ml contained 35 mM Tris-HCl, pH 8, 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 0.01% bovine serum albumin, 0.64 mg/20 ml supercoiled pBr 322 DNA, 1.5 U human topoisomerase I and 1 ml of test compound diluted with DMSO. The reaction mixture was incubated for 30 min at 37ºC. Then, 3 U of Proteinase K was added and the mix was incubated for 45 min at 37ºC again. Finally, the mix was stopped with cold for 15 min and by addition of 5 ml of a mixture of 0.1% bromophenol blue, and 25% glycerol. Inhibition positive controls were performed using camptothecin. Reaction products were submitted to 45 min electrophoresis on a 1% agarose gel in TAE (Tris-acetate-EDTA) running buffer at 60 V. Gels were stained with ethidium bromide (0.50 mg/ml) for 60 min. IC_{50} values were determinate by interpolation from plots of enzyme activity vs. inhibitor concentration. The IC_{50} values are means from at least three independent experiments and standard deviation never exceeded 20%.

CONCLUSION

In summary, one compound (**4**) was found to have excellent inhibitory activity against human topoisomerase I and *Taq* DNA polymerase. This compound can be a leading molecule for the development of original anticancer treatment and an excellent instrument to explore DNA polymerase and topoisomerase activity.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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