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Chiral Salicyl Diamines: Potent Anticancer Molecules

Jian Gao,*[a, b] Ya-Guang Liu,[b] Yaqing Zhou,[b] and Ralph A. Zingaro*[a]

*A set of 12 enantiomeric diamine-based small molecules was synthesized and screened for anticancer activity against five human cancer cell lines: NCI-H460, A549, MCF-7, SK-BR-3, and T-47D. The salicyl diamino compounds (**1**–**6**) were found to induce inhibition of the growth of cancer cells at submicromolar concentrations. The lead compound,* N,N′*-bis-salicyl-(1*R,2R*)-diaminocyclohexane (**1**) displayed single-reagent anticancer activity with an IC_{50} value equal to or less than 2.0 μM in H460 and A-549 cancer cells. SRB and colony formation assays indicated that compound **1** shows greater cytotoxic activity toward MCF-7 cells than MCF-10A cells. Real-time RT-PCR analysis demonstrated that compound **1**, is an extremely efficient regulator of antiapoptotic genes, Bcl-xL, Bcl-2, and the cell cycle related gene, cyclin D1. This study provides a new insight into the development of novel small molecules in the treatment of human breast cancers.*

Introduction

A great challenge for chemists is to identify small molecules which can be involved in the functions of human proteins.[1,2] The discovery of small molecules that either inhibit or activate the protein functions to which they bind is of great importance for the identification of compounds capable of killing cancer cells.[3,4] Bcl-2 family proteins are central regulators of programmed cell death, and members that inhibit apoptosis, such as Bcl-2 and Bcl-xL, are overexpressed in many cancers and contribute to tumor initiation, progression, and resistance to therapy.[5,6] Among all the genes studied to date in the NCI panel of 60 human tumor cell-lines, Bcl-xL shows the strongest correlation with resistance to cytotoxic drugs.[7,8] Recently, a number of small-molecule inhibitors of Bcl-xL and Bcl-2 have been discovered using structure-based design and parallel synthesis.[9,10]

Another meaningful challenge for chemists involves the identification of small molecules that can effectively control the transcription of encoded genes in cancer cells.[11,12] Small molecules which have the capability of interfering directly with the transcription machinery of Bcl-2 family genes may have great potential, not only as cancer therapeutic agents, but also as research tools in chemical biology.[13] An effective small molecule may bind directly to a particular region of DNA or target transcriptional proteins to function. To date, rational design of molecules, which are capable of regulating mRNA expression selectively in cancer cells is still in its infancy.[14] New structural insight and the exploitation of a vast majority of many potent compounds are needed in this area.

Our previous investigation indicated that zinc complexes, which are constructed from chiral diaminocyclohexane and salicyl aldehyde are effective regulators of Bcl-2 genes in cancer cells.[15] A review of the literature indicates that (*R*,*R*)-diaminocyclohexane-bearing compounds is a family of effective anticancer agents.[16,17] There also exist a number of well-documented amino group-bearing compounds, which can interact with DNA[18] or RNA[19] as antibiotics. New evidence has accumulated which shows that polyamino-based compounds might be effective in binding to a particular region of DNA, and hence regulate mRNA expression in human breast cancer cells.[20] It is of particular interest to find more potent diamine-based scaffolds as anticancer agents and to evaluate their potential in regulating Bcl-2 family genes expression in breast cancer cells. Herein, we wish to report the chemical and biological studies of a new class of potential therapeutic agents, chiral salicyl diamines.

Synthetic Chemistry and Cell-line Screening

The synthesis and application of chiral salicyl diamino ligands have been reported in the domain of asymmetric catalysis.[21,22] Using a modified approach, a group of 12 potential drug candidates has been prepared by a two-step synthesis: the Schiff-base condensation and reductive hydrogenation. These chiral diamine-based compounds have been synthesized in good purity and in high yield, without the use of tedious purification procedures. The reactivity between the screened aldehydes and chiral amines (Figure 1) and the insolubility of the resulting Schiff-base molecules in Et_2O/EtOH made it possible to isolate and purify the products in a straightforward manner. The reduction products were further purified by gel-column chromatography (MeOH:CH_2Cl_2 = 1:9). The diamines used are all stereogenic isomers of diaminocyclohexane (DACH) and diphe-

[a] *Prof. J. Gao, Prof. R. A. Zingaro*
Department of Chemistry, Texas A&M University
College Station, TX 77843-3255 (USA)
Fax: (+1) 210-372-0896
E-mail: gaojiantamu@yahoo.com

[b] *Prof. J. Gao, Y.-G. Liu, Y. Zhou*
School of Medicine, University of Texas Health Science Center
San Antonio, TX 78229-3900 (USA)

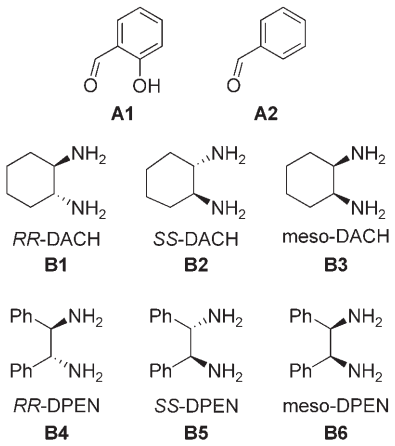

Figure 1. Building blocks in the parallel synthesis of the small molecules.

Table 1. IC_{50} obtained for compounds **1**–**12**.[a]

Compd	NCI-H460, IC_{50} [μM]	A 549, IC_{50} [μM]
1	0.8 ± 0.1	2.1 ± 0.2
2	1.0 ± 0.2	2.3 ± 0.2
3	1.1 ± 0.1	2.6 ± 0.3
4	1.2 ± 0.2	2.4 ± 0.2
5	1.4 ± 0.1	2.5 ± 0.2
6	1.8 ± 0.1	3.1 ± 0.1
7	17.1 ± 0.1	10.8 ± 0.2
8	18.6 ± 0.1	12.5 ± 0.1
9	19.5 ± 0.2	12.9 ± 0.2
10	18.4 ± 0.1	14.4 ± 0.1
11	20.5 ± 0.2	15.0 ± 0.2
12	21.8 ± 0.1	15.8 ± 0.2

[a] Mean values (± SD).

nylethylenediamine (DPEN), which will allow us to identify the stereogenic selectivity of the drug molecules in the in vitro and molecular biological studies.

The reactions between aryl aldehydes (**A1**, **A2**) and the diamines (**B1**–**B6**) resulted in the formation of 12 structurally-related compounds (Figure 2). To evaluate their cytotoxicity, these compounds were evaluated in in vitro screening against NCI-H460 non-small lung and A549 cancer cell lines. The compounds were screened over the range of 100–0.01 μM (5-log dose range) concentrations. We found that the salicyl diamines (**1**–**6**) display higher biological activities, inhibiting cell growth at submicromolar concentrations (Table 1). The IC_{50} values observed for compounds **1**–**6** fall in the range of 0.8–1.8 μM, which is more promising than those compounds with benzoic moieties (**7**–**12**). Compounds with a DACH building block are more active than those with DPEN, which indicates that the cyclohexyldiamine diphenolic moiety represent a useful scaffold in cytotoxic activity. Furthermore, the *R,R* enantiomers seem to be more active than their *S,S* and *R,S* (meso) counterparts.

In Vitro Anticancer Activities Against Breast Cancer Cells and Selective Cytotoxicity

The lead compounds **1**–**3** were further investigated against a series of human breast cancer cell lines. Their anticancer activities against MCF-7 cells were measured using the SRB assay. The percentage of cell survival at a drug concentration of 1.0 μM is displayed in Figure 3a. It is to be noted that com-

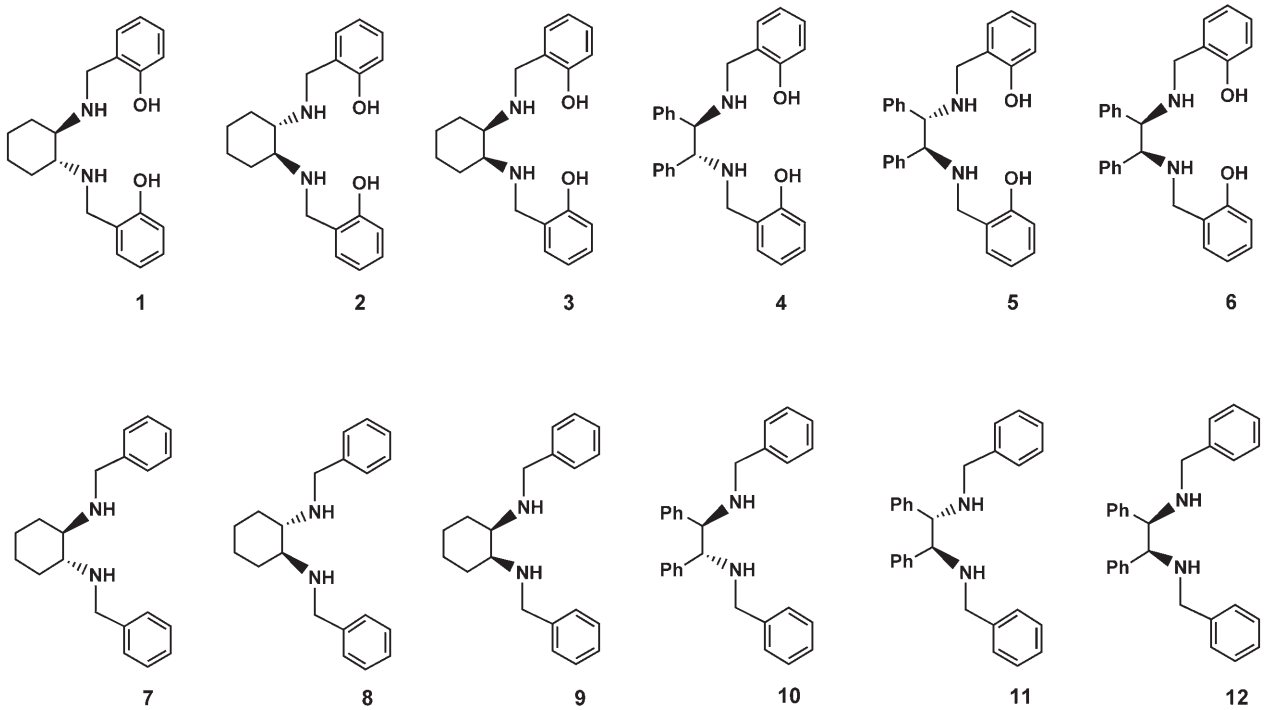

Figure 2. The molecular structure of synthetic compound **1**–**12**.

pound **1** is more active as compared with its stereoisomers, compounds **2** and **3**. For example, at 1.0 μM compound **1** inhibited cell growth by 55%, whereas compound **2** and **3** showed 40% and 25% growth inhibition. To substantiate our observations, these compounds were further tested against two additional human cancer cell lines, that is, SK-BR-3 and T-47D. Against these cells, significant anticancer activities were also observed (Figure 3b and c). It was determined that compound **1** is the most active in the growth inhibition of these three breast cancer cell lines.

The cytotoxicity of compounds **1**, **2**, and **3** was also evaluated against normal human breast cells, MCF-10A using the SRB assay. As is shown in Figure 3d, compound **1** is significantly less toxic against MCF-10A cells as compared with compound **3** at the same concentration. The IC_{50} values determined for MCF-10A cells along with those observed for MCF-7 cells are listed in Table 2. It is evident that the sensitivity of the two cell lines towards compounds **1**, **2**, and **3** varies. Compound **1** was the most toxic toward MCF-7 cells, but it is the least toxic toward MCF-10A compared with compounds **2** and **3**. Based on IC_{50} values, MCF-7 cells were 50, 29, 7-fold more sensitive toward compound **1**, **2**, and **3** than MCF-10A cells. The preferential cytotoxicity observed with compound **1** suggests that *R*,*R* is the stereogenically preferential confirmation in killing breast cancer cells.

Table 2. IC_{50} values for compounds **1**–**3**.[a]

Compd	MCF-7 IC_{50} [μM]	MCF-10A IC_{50} [μM]	Cytotoxicity ratio (MCF-10A/MCF-7)
1	0.4 ± 0.1	19.8 ± 0.1	50
2	0.6 ± 0.1	17.6 ± 0.1	29
3	1.8 ± 0.1	12.7 ± 0.1	7

[a] Mean of two experiments ± range.

Cell Colony Formation Assay

In the colony formation assay, cultured MCF-7 and MCF-10A cells were treated concurrently with compounds **1** to **3** at the concentrations shown (Figure 4a–d). The colony-counting method, in which the cells were treated for a comparatively

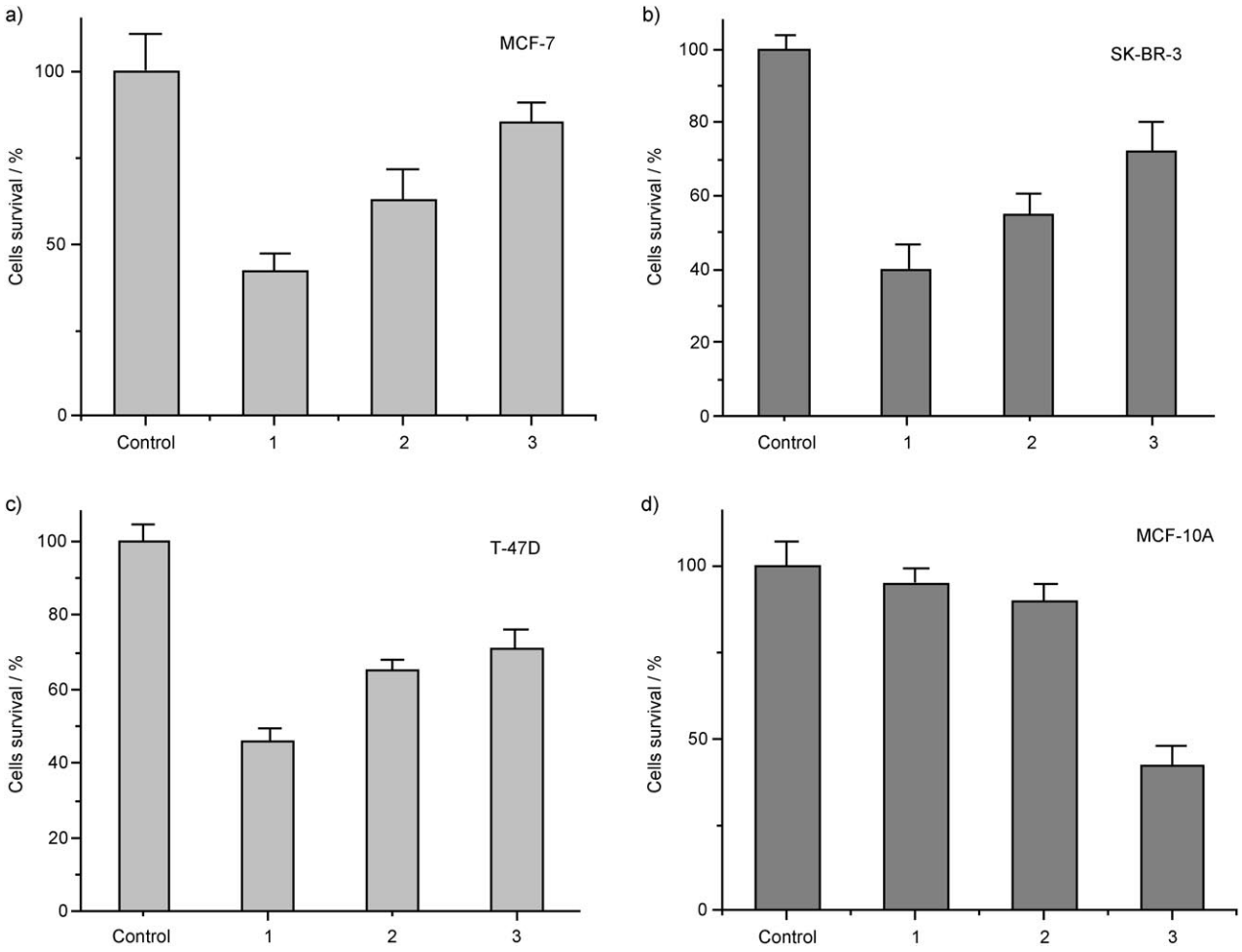

Figure 3. Growth inhibitory effects of compounds **1**–**3** on breast cancer and normal cells-lines. The cells were seeded in 96-well plates and treated at 1.0 μM concentrations for 48 h and the cell survival was measured by SRB assay. The percentage of cell survival for the untreated and treated a) MCF-7 cells, b) SKBR-3, c) T47D, and d) MCF-10A at a concentration of 1.0 μM.

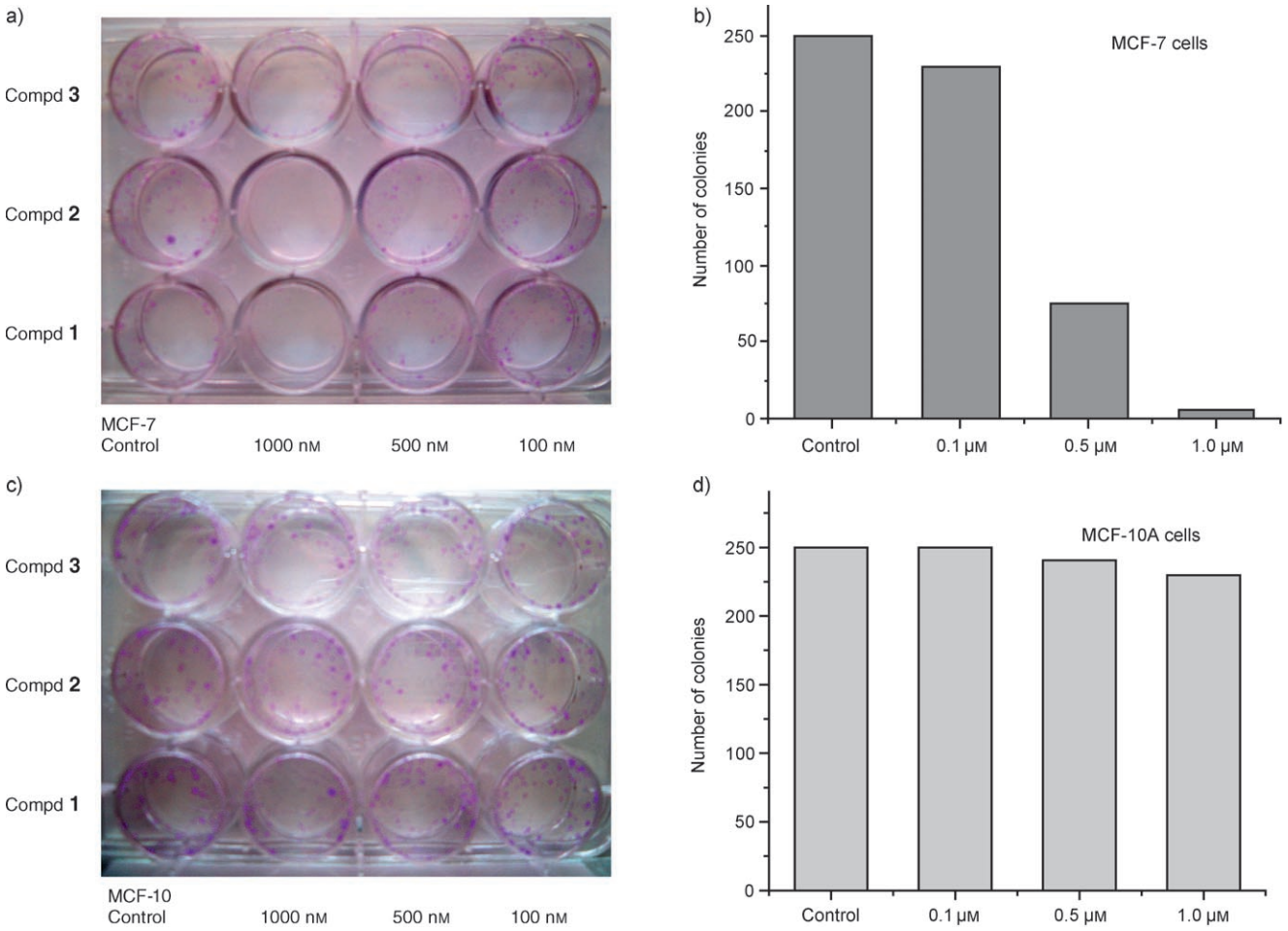

Figure 4. a) MCF-7 cells at a density of ≈250 cells per well were seeded in 12-well plates. On the second day, cells were treated with the indicated concentration of compounds **1**–**3**. The same treatments were repeated every 3 days. After 10 days, the plate was stained for the formation of cell colonies with SRB dye. The picture of the colonies was then taken using a digital camera. b) Number of colonies for the untreated and treated cells at different concentrations of **1**. c) MCF-10A cells at a density of ≈250 cells per well were seeded in 12-well plates. On the second day, cells were treated with the indicated concentration of **1**–**3**. The same treatments were repeated every 3 days. After 10 days, the plate was stained for the formation of cell colonies with SRB dye. The picture of the colonies was then taken using a digital camera. d) Number of colonies for the untreated and treated cells at different concentrations of **1**.

long time than in the SRB inhibition assay, afford more reliable data with respect to the dose level. As is shown in Figure 4a, compound **1** at 1.0 μM not only decreased the sizes of the colonies, but also significantly reduced the number of MCF-7 cell colonies. On the other hand, MCF-10A cells (Figure 4c) are not sensitive to compounds **1**–**3** at the same dose levels. The dose level displayed in this assay is comparable to that determined from the SRB inhibition assay. These results indicate that compound **1** is the most promising candidate against the growth of breast cancer cells.

Inhibition of mRNA Expression in MCF-7 Cells

Cyclins are activators of cyclic-dependent kinases cdk4 and cdk6, which function to translate a growth signal into the cell cycle progress. As a known regulator of cell cycle, cyclin D1 is commonly elevated in breast cancer cells. A number of anticancer pharmaceuticals have been shown to inhibit the cell cycle in various cell types. In this study, the effect of compounds **1**–**3** on the cell cycle of MCF7 was assessed by means of real-time RT-PCR (Figure 5). MCF7 cells were treated with the compounds for 20 h at the same dose (1.0 μM) that induced apoptosis. Compound **1** was found to be the most effective species in the downregulation of cyclin D1 mRNA expression to 40% of the level of untreated cells. Compounds **2** and **3** decreased cyclin D1 mRNA expression to 68% and 71%, respectively. To further address the role of the compounds in the cell cycle progress, we examined whether the overexpression of P27, which is a CDK inhibitor, was induced in the treated cells. Compounds **1**, **2**, and **3** were capable of downregulating the expression of P27 to 41, 13, and 39% of control, respectively. These results suggest that the apoptotic effect of the compounds is unlikely to be a direct outcome of the regulation of cell cycle-related genes.

As discussed in the introduction, Bcl-2 family proteins are central regulators of programmed cell death, and members that inhibit apoptosis, such as Bcl-2 and Bcl-xL, are overexpressed in many cancers and attribute to tumor initiation, pro-

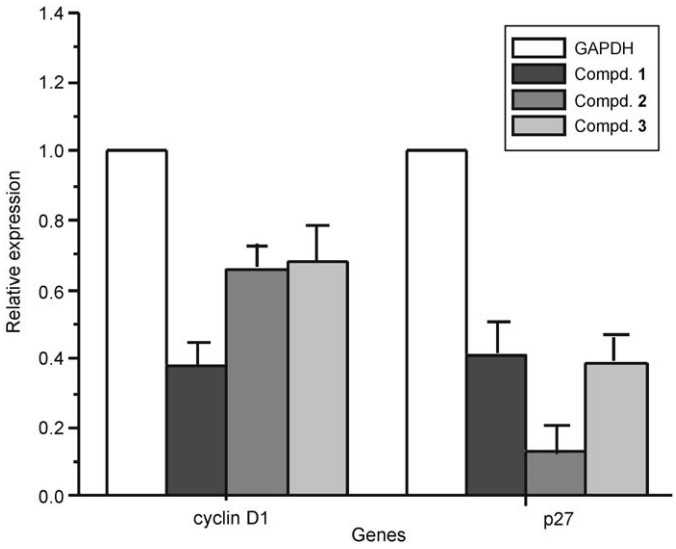

Figure 5. Relative expression of cell cycle related genes. MCF-7 cells were treated with 1.0 μM of compounds **1**–**3** for 24 h. Cyclin D1 and P27 mRNA expression was determined by real-time RT-PCR and normalized with GAPDH expression in each sample.

gression, and resistance to therapy. Hence validation of these gene expressions is critically important to drug discovery. To determine the effects of compounds **1**–**3** on endogenous Bcl-2 mRNA expression, the amount of Bcl-2 mRNA expression in each sample was determined by real-time RT-PCR. As is shown in Figure 6, compound **1** decreased Bcl-xL mRNA expression up to 95% as compared with the level of the untreated cells, and **2** decreased the expression to 20% of the level of the untreated cells. On the other hand, **3** upregulates expression to 130% of control. The antiapoptotic gene Bcl-2 was also examined. Compounds **1** and **2** repress the expression of Bcl-2 to 35% and 55% of control levels. However, **3** overexpressed Bcl-2 to 156% of the control level.

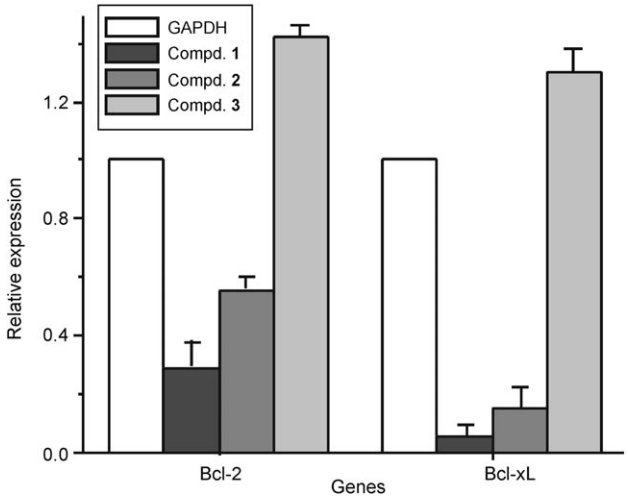

Figure 6. Relative expression of apoptosis related genes. MCF-7 cells were treated with 1.0 μM of compounds **1**–**3** for 24 h. Bcl-2 and Bcl-xL mRNA expression were determined by real-time RT-PCR and normalized with GAPDH expression in each sample.

Discussion

The goal of this study is to identify new anticancer drug molecules that exert their function by regulation of mRNA expression. In the life cycle, biological information flows through macromolecules from DNA to RNA to proteins. Small molecule–RNA interactions plays a key role in this process. In nature, macromolecules reside within their cellular confines. A review of the literature indicates that natural and synthetic small molecules have served as powerful tools in medicine through their interaction with proteins. The discovery of small molecules, which are capable of regulating mRNA expression of oncogenes in cancer cells, is a relatively new event. Furthermore, the innovations of finding small molecules which are capable of inducing apoptosis by manipulation of Bcl-2 family gene expression represent a new approach in the treatment of cancer. Our experimental results indicate that chiral salicyl diamines represent a family of potent drug candidates in this endeavor.

As shown in Figure 7, the binding of small molecules to DNA, transcription factors or coactivators can lead to the destabilization of the multiple-protein assembly, reduce RNA Pol II recruitment, and hence lower the expression of the encoded gene This new concept differs from that for targeting the expressed proteins (passway I in Figure 7) and it has been experimentally validated in this work. During the course of these events, small-molecule modulators can either activate or repress the expression of targeted genes. The real-time RT-PCR results support the concept that this could be the case. The outcome of this investigation is of potential toward the development of small-molecule modulators that could regulate the expression of affected genes (tumor suppressors) to restore normal cell regulation and prevent the uncontrolled growth of cancer cells.

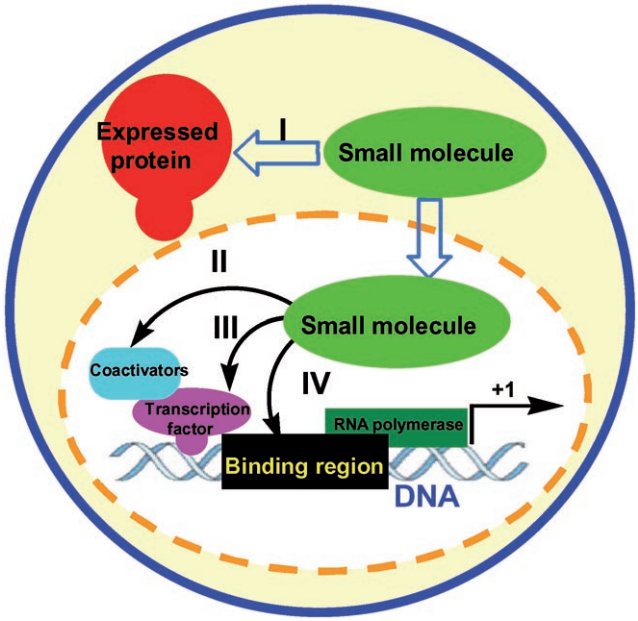

Figure 7. Simplified illustration of the interaction between small molecules with the transcription–initiation complex.

Conclusion

The present study furnishes evidence that *N*,*N*′-bis-salicyl diaminocyclohexanes display carcinostatic activities. SRB cell apoptosis assay and colony formation assays show that (*R*,*R*)-bis-salicyl cyclohexyldiamine is preferentially cytotoxic toward cancer cells. Downregulation of Bcl-2 mRNA expression were observed in MCF-7 cells. The in-depth understanding of the anticancer mechanism of this group of compounds awaits future results from this and other laboratories.

Experimental Section

Materials and measurements. All chemical reagents and solvents were of analytical grade and obtained from the Sigma-Aldrich Chemical Company. Methanol and acetonitrile were dried over molecular sieves (4 Å) prior to use. Analysis for C, H, and N were carried out on a Perkin-Elmer analyzer, Model 240. Positive ion ESI-MS spectra were recorded using LCQ electrospray mass spectrometer. The spectra were recorded over the mass range *m*/*z* 200–1000.

Synthesis of compounds 1–3. For the synthesis of compound **1**, a solution of 2-hydroxy-benzaldehyde (0.20 mmol) was added dropwise to a solution containing (*R*,*R*)-DACH (0.1 mmol) in 10 mL of EtOH, and this was followed by the addition of 0.10 mmol of Et_3N to the system. After 2 h of stirring, a yellow-colored Schiff-base product precipitated from the solvent and this was separated by filtration. The resulting Schiff-base was dissolved in ethanol/acetonitrile, and to this $NaBH_4$ (0.2 mmol, 80 mg) was added in solid form to reduce the Schiff-base. The solution was magnetically stirred for an additional 4 h at RT. The solvent was removed using a rotary evaporator. The reduced product was purified by column chromatography and was identified as the desired product. Yield 82%, Anal. Calcd for $C_{20}H_{26}N_2O_2$, C 73.6, H 8.03, N 8.58. Found: C 73.2, H 8.00, N 8.59%. $[\alpha]_D^{25} = -26.6^\circ$ ($c = 0.01$, CH_2Cl_2). ESI-MS calculated for $[C_{20}H_{26}N_2O_2+H]^+$ 362.4, found 362.4. ^{1}H NMR ($CDCl_3$): $\delta =$ 1.29–1.45 (m, 8H, CH_2 of cyclohexane), 3.12 (s, 2H, CH of cyclohexane), 3.84 (m, 4H, CH_2-Ar), 6.52–7.03 ppm (m, 8H, H-Ar). Compounds **2** and **3** were synthesized from (*S*,*S*)- or (*R*,*S*)-DACH by using a similar procedure to that described and were characterized by elemental analysis, ESI-MS, and ^{1}H NMR.

Synthesis of compounds 4–6. For the synthesis of compound **4**, a solution of 2-hydroxy-benzaldehyde (0.20 mmol) was added dropwise to a solution containing (*R*,*R*)-DPEN (0.1 mmol) in 10 mL of EtOH, and this was followed by the addition of 0.10 mmol of Et_3N to the system. After 2 h of stirring, a yellow-colored Schiff-base product precipitated from the solvent and this was separated by filtration. The resulting Schiff-base was dissolved in ethanol/acetonitrile and to this $NaBH_4$ (0.2 mmol, 80 mg) was added in solid form to reduce the Schiff-base. The solution was magnetically stirred for an additional 4 h at RT. The solvent was removed using a rotary evaporator. The reduced product was purified by column chromatography and was identified as the desired product. Yield 84%, Anal. Calcd for $C_{28}H_{28}N_2O_2$, C 79.2, H 6.65, N 6.60. Found: C 79.2, H 6.89, N 6.42%. $[\alpha]_D^{25} = +48.6^\circ$ ($c = 0.01$, CH_2Cl_2). ESI-MS calculated for $[C_{28}H_{28}N_2O_2+H]^+$ 425.5, found 425.6. ^{1}H NMR ($CDCl_3$): $\delta = 3.80$ (s, 4H, CH_2 of CH_2-Ar-OH), 6.61–7.25 ppm (m, 18H, H-Ar). Compounds **5** and **6** were synthesized from (*S*,*S*)- or (*R*,*S*)-DPEN by using a similar procedure to that described and were characterized by elemental analysis, ESI-MS, and ^{1}H NMR.

Synthesis of compounds 7–9. For the synthesis of compound **7**, a solution of benzaldehyde (0.20 mmol) was added dropwise to a solution containing (*R*,*R*)-DACH (0.1 mmol) in 10 mL of EtOH. After 2 h of stirring, a yellow-colored Schiff-base product precipitated from the solvent and this was separated by filtration. The resulting Schiff-base was dissolved in ethanol/acetonitrile and to this was added $NaBH_4$ (0.2 mmol, 80 mg) in solid form to reduce the Schiff-base. The solution was magnetically stirred for an additional 4 h at RT. The solvent was removed using a rotary evaporator. The reduced product was purified by column chromatography and was identified as the desired product. Yield 63%, Anal. Calcd for $C_{20}H_{26}N_2$, C 81.6, H 8.90, N 9.51, Found: C 81.6, H 8.53, N 9.60%. $[\alpha]_D^{25} = -28.4^\circ$ ($c = 0.01$, CH_2Cl_2). ESI-MS calculated for $[C_{20}H_{26}N_2+H]^+$ 295.52, found 295.50. ^{1}H NMR ($CDCl_3$): $\delta = 1.32$–1.38 (m, 8H, CH_2 of cyclohexane), 2.85 (s, 2H, CH of cyclohexane), 3.82 (m, 4H, CH_2-thiophene), 7.02–7.22 ppm (m, 10H, H-Ar). Compounds **8** and **9** were synthesized from (*S*,*S*)- or (*R*,*S*)-DACH by using a similar procedure to that described and were characterized by elemental analysis, ESI-MS, and ^{1}H NMR.

Synthesis of compounds 10–12. For the synthesis of compound **10**, a solution of benzaldehyde (0.20 mmol) was added dropwise to a solution containing (*R*,*R*)-DPEN (0.1 mmol) in 10 mL of EtOH. After 2 h of stirring, a yellow-colored Schiff-base product precipitated from the solvent and this was separated by filtration. The resulting Schiff-base was dissolved in ethanol/acetonitrile and to this was added $NaBH_4$ (0.2 mmol, 80 mg) in solid form to reduce the Schiff-base. The solution was magnetically stirred for an additional 4 h at RT. The solvent was removed using a rotary evaporator. The reduced product was purified by column chromatography and was identified as the desired product. Yield 77%, Anal. Calcd for $C_{28}H_{28}N_2$, C 85.7, H 7.19, N 7.14. Found: C 85.7, H 7.21, N 6.92%. $[\alpha]_D^{25} = +46.9^\circ$ ($c = 0.01$, CH_2Cl_2). ESI-MS calculated for $[C_{28}H_{28}N_2+H]^+$ 393.53, found 393.62. ^{1}H NMR ($CDCl_3$): $\delta = 3.80$ (s, 4H, CH_2-Ar), 4.51 (s, 2H, CH-Ar), 7.11–7.29 ppm (m, 20H, H-Ar). Compounds **11** and **12** were synthesized from (*S*,*S*)- or (*R*,*S*)-DPEN by using a similar procedure to that described and were characterized by elemental analysis, ESI-MS, and ^{1}H NMR.

Cell culture. Human breast cancer cell lines MCF-7, SKBR-3, T-47D, NCI-H460, A549, and MCF-10A were obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in RPMI medium supplemented with 10% FBS, penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in an atmosphere humidified with 5% CO_2 and 95% air.

Growth inhibition assay. Cells were seeded in 96-well cell culture plates and treated on the second day with the drug candidates. At the end of a 2 day treatment, the cell number was measured by the SRB assay as described in the literature.[23] The percentage of growth inhibition was calculated by using the equation: % growth inhibition $= (1 - A_t/A_c)\,100\%$, where A_t and A_c represent the absorbance in treated and control cultures, respectively.

Colony formation assay. Cells (single-cells suspension) were plated into 12-well plates at a density of 200–300 cells per well. On the second day, cells were treated with compounds **1**–**3**. Every three days, the medium was replaced with fresh medium containing the agent with corresponding concentrations. After a ten-day treatment, the medium was removed and cell colonies were stained with SRB dyes according to the company protocol (Sigma). Pictures were taken using a digital camera.

Real-time RT-PCR analysis. Total RNA from MCF-7 cells was isolated, following homogenization of the tissue, with the Tri Reagent (Sigma, St. Louis, MO). After DNA-free™ DNase treatment (Ambion,

Austin, TX), 200 ng of the total RNA was used in reverse transcription (RT) by the GeneAmp RNA PCR kit (Perkin-Elmer, Foster City, CA), Real-time PCR was then performed on cDNA from RT reactions, using OmniMix HS bead (Cepheid, Sunnyvale, CA) according to the manufacturer's protocol. SYBR green dye (1×; Fisher Scientific; Atlanta, GA) was added to the reaction mixture to detect amplicon synthesis in the SmartCycler real-time PCR thermal cycler (Cepheid, Sunnyvale, CA). Specific primers of genes were designed on different exons, the following primers were used: Cyclin D1, forward 5′-ATGGAACACCAGCTCCTGTGCT-3′, reverse 5′-GCGGCCAGGTTCCACTTGAGCT-3′; P27: forward 5′-CTGTGGAGCAGACGCCCAAGAAG-3′, reverse 5′-CCTGCCCTCCCTTCCCCAAAGTT-3′; Bcl-2: forward 5′-CAGCTGCACCTGACGCCCTTCACC-3′, reverse 5′-CTGAGCAGAGTCTTCAGAGACAGC-3′; Bcl-xL: forward 5′-GCACTGTGCGTGGAAAGCGTAGAC-3′, reverse 5′-CTGAAGAGTGAGCCCAGCAGAACC-3′; The specificity of the PCR amplified product was verified by sequencing the product (data not shown). The thermal cycling conditions included an initial denaturation step at 95 °C for 2 min and 45 cycles at 95 °C for 10 s, 65 °C for 15 s and 72 °C for 30 s. For quantification, the cycle threshold number (C_t) that exhibits the maximum curve growth rates was determined using the Cepheid SmartCycler software. The relative gene expression of each sample, normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was calculated by the formula $2^{c_t(\mathrm{GAPDH})-c_t(\mathrm{gene})}$.

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Keywords: Bcl-xL expression · chiral cyclohexyldiamines · cytotoxic activity · human breast cancer cells · small molecules

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