A Novel Achiral *seco*-Amino-Cyclopropylindoline (CI) Analog of CC-1065 and the Duocarmycins: Design, Synthesis and Biological Studies

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Abstract: The design, synthesis and DNA binding properties of a novel achiral and amino-containing *seco*-cyclopropylindoline analog (*seco*-amino-CI-TMI, **1**) of the duocarmycins are described. Thermal induced DNA cleavage studies on pUC18 DNA revealed compound **1** to preferentially bind in the minor groove and to covalently react with AT-rich sequences, particularly at the underlined adenine-N3 group of 5'-AAAAA(865)-3'. This sequence specificity is similar to adozelesin and CC-1065. Using a 4-day continuous exposure, compound **1** inhibited the growth of K562 human chronic myeloid leukemia cells in culture. Compound **1** has appreciable cytotoxicity (IC_{50} value of 1.30 μM) relative to compound **2** (0.15 μM), the corresponding racemic and hydroxy-*seco*-CI-TMI analog. These results indicate that the aminophenethyl chloride group present in compound **1** has similar sequence specific and cytotoxic properties to the hydroxy-containing *seco*-precursors of CC-1065 and the duocarmycins. Moreover, the results suggest that the chiral center present in the natural products is not absolutely necessary for biological activity. The novel aminophenethyl halide moiety is, therefore, a useful template from which to develop future achiral analogs of CC-1065 and the duocarmycins.

Key Words: CC-1065, duocarmycins, DNA alkylation, sequence specificity, cytotoxicity.

INTRODUCTION

CC-1065 (**3**) and the duocarmycins, exemplified by duocarmycin SA (**4**) and depicted in Figure **1**, are potent anticancer agents [1]. These compounds contain a cyclopropylpyrroloindolone (CPI) DNA alkylating pharmacophore, and they derive their potent cytotoxicity through alkylation of adenine-N3 groups in the minor groove of 5'-PuNTTA-3' and 5'-AAAAA-3' sequences [1]. Several analogs, adozelesin [2], carzelesin [3], bizelesin [4], and KW2189 [5] have entered clinical evaluation, but only bizelesin is still undergoing phase II trial [4]. Clinical studies on the other three compounds were discontinued due to their adverse toxicity, particularly to bone marrow [2,3,5].

As a consequence, there has been a strong interest in the design and development of novel analogs of CC-1065 and the duocarmycins that effectively kill cancer cells and have reduced toxicity to the host. One class of analogs of CC-1065 and the duocarmycins that have received significant attention contain an amino group in place of the hydroxyl moiety in the *seco*-prodrug congeners of CC-1065 and duocarmycin SA. The chiral *seco*-amino-CI-TMI (**5**) [6] and *seco*-amino-cyclopropylbenzoindoline-TMI (*seco*-amino-CBI-TMI, **6**) [7] analogs have been demonstrated to have comparable DNA alkylation, sequence specificity, and anticancer activities to their hydroxyl counterparts. In the search for less toxic analogs of the duocarmycins, we have recently reported that *seco*-iso-CFI-TMI (iso-cyclopropylfuranoindoline) analog **7** is endowed with potent anticancer activity and it is relatively non-toxic to murine bone marrow cells, when compared to *seco*-CBI-TMI **8** [8].

As part of our efforts in the design of novel analogs of CC-1065 and the duocarmycins, our group has embarked on a program to investigate whether the chiral center present in the natural products is needed for biological activity. To date, there has been little activity on the development of achiral analogs of CC-1065 and the duocarmycins. To our knowledge, two papers on non-chiral analogs have been reported. One report describes the synthesis and DNA binding studies of a bis-chloromethyl *seco*- CBI-TMI analog [9]. The bulky prochiral molecule was found to poorly alkylate DNA and ineffectively produce interstrand crosslinks, leading to weak cytotoxicity compared to *seco*-CBI-TMI **8**. The other report detailed the synthesis of a spiro[2,5]cyclopropanecyclohexadienone derivative, but no biochemical studies were conducted [10]. As part of our studies on achiral analogs of CC-1065 and the duocarmycins, we hypothesized that the chiral center present in the structures is not necessary for biological activity. Accordingly, as illustrated in Figure **2**, we have designed an achiral analog **1** (*seco*-amino-CI-TMI) that contains a 4-aminophenethyl halide functionality. Similar to the loss of HCl in *seco*-CC-1065 and *seco*-duocarmycin compounds in biological media, compound **1** should lose HCl to produce a putative spiro[2,5]cyclopropanecyclohexadienimine DNA alkylating agent **9**, which reacts with an adenine-N3 group in the minor groove of AT-rich sequences. It is worthy to note

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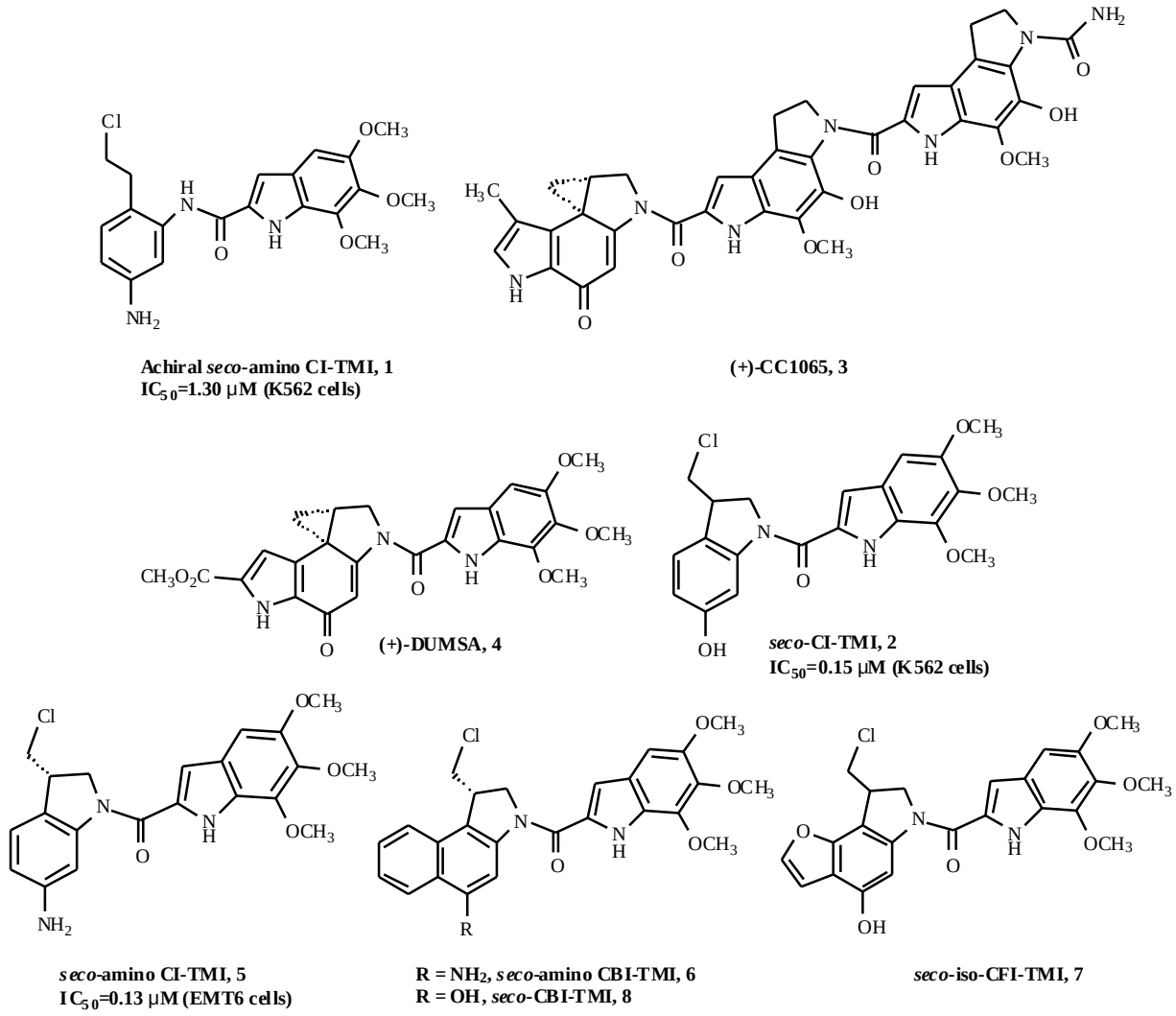


Fig. (1). Structures of the target compound **1**, *seco*-CI-TMI (**2**), (+)-CC1065 (**3**), (+)-DUMSA (**4**), *seco*-amino-CI-TMI (**5**), *seco*-amino CBI-TMI (**6**), *seco*-iso-cyclopropylfuranoindoline (CFI)-TMI (**7**), and *seco*-cyclopropylbenzoindoline (CBI)-TMI (**8**). TMI = 5,6,7-trimethoxyindole-2-carbonyl.

that the corresponding hydroxyphenethyl halides are known to readily lose hydrogen halide to generate the corresponding spiro[2,5]cyclo-propanecyclohexadienone, which reacts with DNA and possesses cytotoxic properties [11]. In this report, we will describe the synthesis of compound **1** along with its DNA sequence specific alkylation and anticancer cytotoxic properties.

METHODS AND MATERIALS

Melting points were determined on a Mel-Temp apparatus and were uncorrected. The ^{1}H- NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer. The appropriate deuterated solvents are indicated in the procedure, with TMS as the internal standard and line positions are recorded in ppm from the reference signal. Infared spectra were recorded on a Perkin Elmer Paragon 500 FT-spectrophotometer and only the principle sharply defined bands were reported in cm^{-1}. Mass spectra and accurate mass measurements were recorded at the University of South Carolina. Elemental analyses were performed at Midwest Microlabs, Indiana.

Commercial grade solvents and reagents were used without further purification with the following exceptions: triethylamine, aceto-nitrile, acetic anhydride, formic acid, $CDCl_3$, pyridine, methanol, and deuterated DMSO-d_6 were dried over molecular sieves 3Å. THF was dried by distillation over sodium and benzophenone. DMSO was distilled over NaOH under vacuum and stored over molecular sieves 3Å. DMF was distilled over BaO under vacuum and stored over molecular sieves 3Å.

N-benzyl-4-chloro-3-nitroaniline 11

Two equal batches of 4-chloro-3-nitroaniline **10** (20.0 g each, 0.116 mol) were dissolved separately in freshly distilled dichloromethane (over P_2O_5) (150 mL each) and dry triethylamine (1.1 eq. each, 18 mL, 0.128 mol) under a

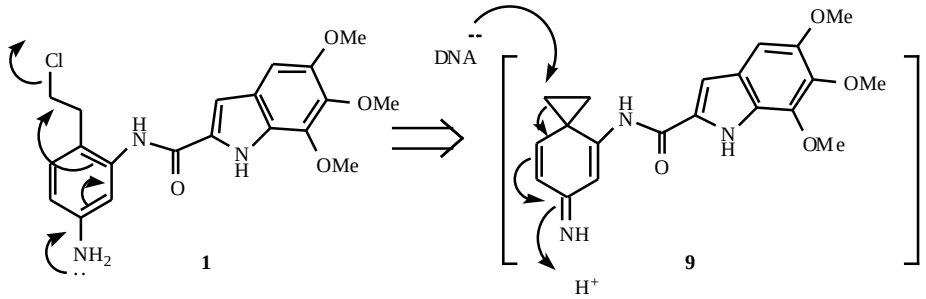


Fig. (2). Proposed mechanism of activation and DNA alkylation of the achiral *seco*-amino-CI moiety in compound **1**.

drierite drying tube. Each of the reaction mixtures was chilled in an ice bath and benzyl chloroformate (50 mL each, 0.348 mol) was slowly added. The resulting solutions were heated to reflux for 4 days, and then they were combined and concentrated. The residue was further concentrated *in vacuo* using a Kugelrohr apparatus (0.1 mm Hg, 60°C) to give a viscous oil. The oily material was dissolved in chloroform (300 mL) and washed with brine (100 mL). The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified on a silica gel column, using 2.5% methanol/chloroform as a solvent. A total yield of 32.2 g (53% yield) of N-benzyloxycarbonyl-4-chloro-3-nitroaniline **11** was isolated as a yellow oil that solidified upon refrigeration. R_f = 0.61 (20% ethyl acetate-hexane). IR (neat) 3424, 3100, 3060, 3025, 1603, 1528, 1493, 1448, 1395. ^{1}H -NMR (500 MHz, $CDCl_3$) 7.28 (t, 9.0 Hz, 2H), 7.24 (s br, 1H), 7.22 (t, 9.0 Hz, 1H), 7.16 (d, 8.0 Hz, 1H), 7.13 (d, 9.0 Hz, 2H), 6.98 (d, 3.0 Hz, 1H), 6.73 (dd, 3.0, 8.0 Hz, 1H), 4.60 (s, 2H). ^{13}C-NMR ($CDCl_3$) 148.5, 148.1, 136.6, 132.0, 129.0, 127.5, 126.4, 116.7, 113.2, 108.5, 54.6. EI-MS 263 (M^+, 35%).

Diethyl ((N-benzyl)-3-nitroanilin-4-yl)malonate 12

Sodium hydride (1.5 g of a 60% mineral oil suspension, 0.038 mol) was washed with dry hexane and suspended in dry dimethylsulfoxide (15 mL). The reaction mixture was kept under a nitrogen atmosphere and the flask was chilled in an ice bath. Diethyl malonate (4.0 mL, 0.0325 mol) was slowly added. In a separate flask, N-benzyl-4-chloro-3-nitroaniline 11 (1.0 g, 3.8 mmol) was dissolved in dry dimethylsulfoxide (15 mL), and it was slowly added to the chilled sodium malonate diester reaction mixture. The resulting solution was heated in an oil bath at 105-115 °C for 64 hours. The solvent was removed by distillation using a Kugelrohr apparatus (0.1 mm Hg, 60 °C) to give a viscous residue, which was dissolved with chloroform (300 mL). The organic layer was washed twice with water (150 mL each), and dried over sodium sulfate. Concentration of the organic layer gave an oily residue that was purified by silica gel chromatography, using a 5-10% ethyl acetate/hexane gradient solvent system. The desired product, diethyl ((N-benzyl)-3-nitroanilin-4-yl)malonate 12, was isolated as an orange oil which solidified upon refrigeration (0.88 g, 60%). R_f = 0.44 (20% ethyl acetate-hexanes). IR (neat) 3400, 3061, 3030, 1732, 1623, 1534, 1446, 1394. ^{1}H -NMR (500 MHz, $CDCl_3$) 7.13-7.30 (m, 8H), 6.84 (dd, 3.0, 8.0 Hz, 1H), 4.62 (s, 2H), 4.16 (q, 6.5 Hz, 4H), 3.39 (s, 1H), 1.20 (t, 6.5 Hz, 6H). EI-MS 386 (M^+, 12%).

2-((N-Benzyl)-3-nitroanilin-4-yl)ethanol 13

A solution of diethyl ((N-benzyl)-3-nitroanilin-4-yl)malonate **12** (0.500 g, 1.29 mmol) in ethanol (15 mL) and 10% NaOH (aq) (18 mL) was refluxed for 4 hrs. At that time, the ethanol was removed under vacuum and THF (20 mL) was added. Upon chilling the solution in an ice bath, the solution was adjusted to pH 1 with 6 M HCl. The solution was refluxed for another hour. After the THF was removed from the biphasic mixture, the aqueous layer was extracted with chloroform (2 x 100 mL). The combined organic phases were dried over sodium sulfate then concentrated to a dark brown oil under vacuum. The crude product was purified by silica gel column chromatography using chloroform as solvent to give ((N-benzyl)-3-nitroanilin-4-yl)acetic acid as a thick yellow oily residue (0.35 g, 95%). R_f = 0.29 (10% methanol-chloroform). IR (neat) 3600 – 2600 br, 3025, 2927, 1714, 1630, 1532, 1453, 1399, 1351. ^{1}H -NMR (500 MHz, $CDCl_3$) 11.52 (s br, 1H), 7.95 (s, 1H), 7.42 (d, 3.0 Hz, 1H), 7.27 (t, 8.0 Hz, 2H), 7.21 (t, 7.5 Hz, 1H), 7.16 (d, 7.0 Hz, 2H), 7.01 (d, 8.5 Hz, 1H), 6.84 (dd, 3.0, 8.0 Hz, 1H), 4.63 (s br, 2H), 3.82 (s br, 2H).

((N-Benzyl)-3-nitroanilin-4-yl)acetic acid (0.35 g, 1.22 mmol) was dissolved in dry THF (5 mL) and kept under nitrogen, then the solution was chilled in an ice bath. A solution of borane-THF complex (3.6 mL of a 1.0 M solution, 3.6 mmol) was slowly added, and the mixture was stirred at room temperature for 2.5 hrs. The reaction mixture was quenched with water. The THF layer was removed, and the aqueous phase was extracted with dichloromethane (3 x 100 mL). The combined organic phases were dried over sodium sulfate, then concentrated to an oil under vacuum. The crude product was purified by silica gel column chromatography using a gradient solvent system (chloroform to 5% methanol-chloroform) to afford 2-((N-benzyl)-3-nitroanilin-4-yl)ethanol **13** as a thick colorless oil (0.33 g, 99%.). R_f = 0.59 (10% methanol/chloroform). IR (neat) 3565, 3352, 3060, 3024, 1621, 1528, 1453, 1395. ^{1}H -NMR (500 MHz, $CDCl_3$) 7.27 (t, 7.5 Hz, 2H), 7.22 (t, 7.0 Hz, 1H), 7.20 (s, 1H), 7.19 (d, 3.0 Hz, 1H), 7.16 (d, 7.5 Hz, 2H), 7.07(d, 8.5 Hz,1H), 6.83 (dd, 3.0, 8.5 Hz, 1H), 4.61(s, 2H) 3.80 (t, 6.5 Hz, 2H), 2.93 (t, 6.5 Hz, 2H). FAB-MS 273 ($M+H^+$, 5%).

2-((N-Benzyloxycarbonyl)-3-nitroanilin-4-yl)ethyl Chloride 14

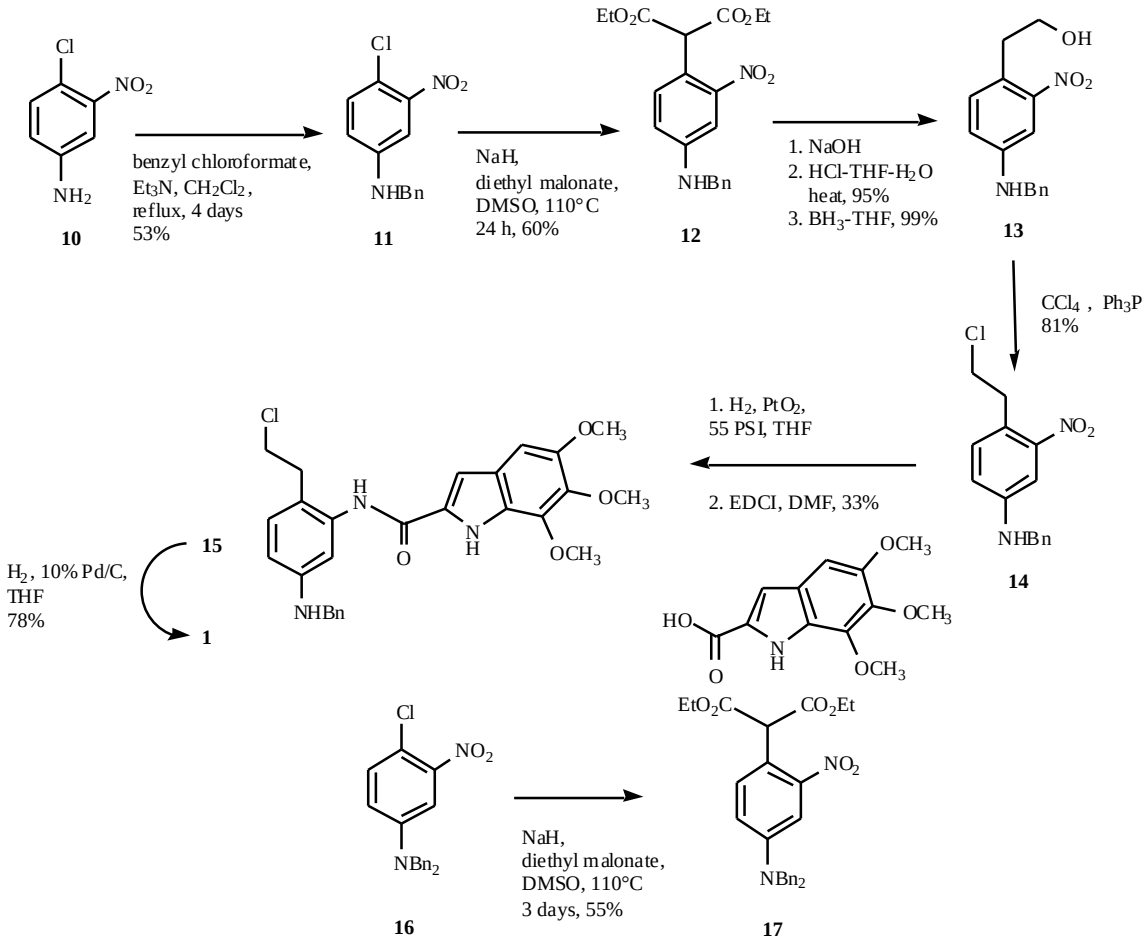
To a solution of 2-((N-benzyloxycarbonyl)-3-nitroanilin-4-yl)ethanol **13** (0.33 g, 1.04 mmol) and triphenyl-phosphine

(0.55 g, 2.09 mmol) in freshly distilled dichloromethane (15 mL) kept under a nitrogen atmosphere, was added carbon tetrachloride (0.6 mL, 3.85 mmol). After the reaction mixture was stirred at room temperature for one day, it was concentrated to an oily residue. Purification of the residue on a silica gel column with chloroform gave 2-((N-benzyloxycarbonyl)-3-nitroanilin-4-yl)ethyl chloride **14** as a thick orange-colored oil (0.27 g, 81%), which solidified upon standing in the refrigerator. R_f = 0.80 (5% methanol-chloroform). IR (neat) 3344, 3060, 3025, 1626, 1528, 1493, 1453, 1399, 1346, 734. ^{1}H -NMR (500 MHz, $CDCl_3$) 7.27 (t, 8.0 Hz, 2H), 7.25 (d, 3.0 Hz, 1H), 7.22 (s, 1H), 7.21 (t, 8.5 Hz, 1H), 7.16 (d, 7.5 Hz, 2H), 7.07 (d, 8.5 Hz, 1H), 6.82 (dd, 3.0, 8.5 Hz, 1H), 4.62 (s, 2H), 3.68 (t, 7.0 Hz, 2H), 3.11 (t, 7.0 Hz, 2H). EI-MS 289 (M^+-H, 10%).

4-(2-chloroethyl)-3-(5,6,7-trimethoxyindole-2-carboxamido)aniline 1

A mixture of compound **14** (1.01 g, 3.48 mmol) with PtO_2 (250 mg) was suspended in chilled THF (60 mL), and the suspension was hydrogenated (with shaking) at 55 PSI and room temperature for one hour. The suspension was filtered over Celite, and the filtrate was concentrated under reduced pressure. The oily residue was co-evaporated with dry CH_2Cl_2 (twice) then kept under vacuum. Because the amine intermediate was unstable, it was used directly in the next step.

A sample of the 3-amino-2-((N-benzyl)anilin-4-yl)ethyl chloride (169 mg, 0.65 mmol) was dissolved in dry CH_2Cl_2 (20 mL). This solution was then added to a stirred suspension of 5,6,7-trimethoxyindole-2-carboxylic acid (150 mg, 0.60 mmol) and benzotriazol-1-yloxy-tripyrrolidinophos-phonium hexafluorophosphate (PyBOP) (296 mg, 0.57 mmol) in dry CH_2Cl_2 (55 mL) under a nitrogen atmosphere. Freshly distilled N,N-diisopropylethylamine (0.22 mL, 1.28 mmol) was added, and the resulting clear solution was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with water (50 mL), 1M HCl (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was then dried over sodium sulfate and concentrated. The residue was purified on a silica gel column using a 2.5% ethyl acetate-dichloromethane solvent system to give 2-(N-benzyl)-3-(5,6,7-trimethoxyindole-2-carbox-amido)anilin-4-yl)ethyl chloride **15** as clear colorless foamy solid (106 mg, 33%). M.p. 70-74 °C. R_f = 0.55 (6% EtOAc-CH_2Cl_2). IR (neat) 3291, 3060, 3007, 1726, 1621, 1577, 1537, 1497, 1461, 1448, 1408, 747. ^{1}H -NMR ($CDCl_3$, 500 MHz) 9.11 (s, 1H), 7.92 (s, 1H), 7.26-7.14 (m, 7H), 6.94



Scheme 1. Synthesis of the target compound **1**, achiral *seco*-amino-CI-TMI.

(d, 8.5 Hz, 1H), 6.74 (s, 1H), 6.66 (s br, 1H), 6.50 (dd, 3.0, 8.5 Hz, 1H), 4.58 (s, 3H), 3.97 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.69 (t, 6.5 Hz, 2H), 2.95 (t, 6.5 Hz, 2H).

Compound **15** (187 mg, 0.37 mmol) was combined with 10% Pd/C (95 mg) and suspended in chilled THF (20 mL). The suspension was purged (three times) and kept under hydrogen at atmospheric pressure and room temperature for three days. The suspension was filtered over Celite, and the yellow solution was concentrated *in vacuo*. The yellow-brown residue was purified on a silica gel column, using a methanol-chloroform gradient (0-6%) solvent system, to give 4-(2-chloroethyl)-3-(5,6,7-trimethoxy-indole-2-carboxamido)aniline **1** (117 mg, 78%) as an oily residue. R_f = 0.21 (3% EtOAc-CH_2Cl_2). IR (neat) 3068, 1723, 1621, 1581, 1536, 1503, 1461, 750. ^{1}H-NMR ($CDCl_3$, 500 MHz) 9.80 (1H, s), 8.22 (1H, s), 7.11 (1H, s), 6.87 (d, 8.5 Hz 1H), 6.85 (1H, s), 6.72 (1H, s), 6.42 (dd, 8.5, 3.0 Hz, 1H), 4.42 (2H, s br), 3.93 (3H, s), 3.84 (3H, s), 3.80 (3H, s), 3.64 (t, 6.5 Hz, 2H), 2.90 (t, 6.5 Hz, 2H). FAB-MS 405 (M^+, 4%). Analysis for $C_{20}H_{22}N_3O_4Cl.H_2O$: calcd. C, 56.94, H, 5.73, N, 9.96; obsd. C, 56.55, H, 6.01, N, 10.22.

Cytotoxicity Studies on Human Chronic Myeloid Leukemia Cells

The K562 human chronic myeloid leukemia cells were maintained in RPM1 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were incubated with a specified dose of drug for 4 days at 37 °C in the dark. The incubation was terminated by centrifugation (5 minutes, 300 g) and the cells were washed once with drug-free medium. Following the appropriate drug treatment, the cells were transferred to 96-well microtitre plates, 10^4 cells per well, 8 wells per sample. Plates were then kept in the dark at 37 °C in a humidified atmosphere containing 5% CO_2. Following incubation of the plates for 4 days (to allow control cells to increase in number by 10 fold) 20 μL of a 5 mg/mL solution of MTT (3-4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide, Sigma Chemical Co.) in phosphate buffered saline was added to each well and the plates further incubated for 5 hours. The plates were then centrifuged for 5 minutes at 300 g and the bulk of the medium pipetted from the cell pellet leaving 10-20 μL per well. 200 μL DMSO was added to each well and the samples agitated to ensure complete mixing. The optical density was then read at a wavelength of 550 nm on a Titertek Multiscan ELISA plate reader, and the dose-response curve was constructed. For each curve, an IC_{50} value was read as the dose required to reduce the final optical density to 50% of the control value.

Thermal Cleavage Studies

The DNA fragment used in these studies was obtained from PCR amplification of base pairs 749-956 of the pUC18 plasmid that was linearized with *Hind III*. A 5'-^{32}P-labeled-TGGTATCTTT-ATAGTCCTGTCG-3' and an unlabelled primer 5'-CTCACTCAAAGGCGGTAATAC-3' were used in the experiment. The labeled DNA fragment was purified by elution through Bio-Rad spin columns and incubated with the compounds for 5 hours at 37°C. The compound-DNA interactions were terminated by precipitation of the DNA, lyophilization of the samples, and re-suspending the pellets in 100 μL of sodium citrate buffer, pH 7.2. Samples were dissolved in formamide loading dye, heated for 3 minutes at 90 °C, cooled on ice, and electrophoresed at 2500-3000V for 3 h on a 80 cm x 20 cm x 0.4 mm 6% acrylamide denaturing sequencing gel (Sequagel, National Diagnostics). The gels were dried, and x-ray film was exposed to the gels (Hyperfilm, Amersham. UK). Densitometry was carried out on a Bio-Rad GS-670 imaging densitometer.

RESULTS AND DISCUSSION

The synthesis of the target achiral *seco*-amino-CI-TMI compound **1** began with protection of the amino functionality of 4-chloro-3-nitroaniline **10**. Reaction of amine **10** with benzyl chloroformate in refluxing dichloromethane did not produce the benzyloxycarbonyl or "Z" protected amine, instead it predominantly gave the N- benzylated amine product **11** [12]. Compound **11** was isolated as a yellow solid product in 53% yield, after silica gel column chromatography. The presence of a benzyl protecting group in compound **11**, instead of a Z moiety, was ascertained by mass spectrometry, ^{1}H-NMR and ^{13}C-NMR methods. A molecular ion of 263 amu in an electron impact mass spectrum, a signal for the benzylic hydrogens at 4.60 ppm in the ^{1}H-NMR spectrum, and a benzylic-^{13}C signal at 54.6 ppm in the ^{13}C-NMR spectrum were consistent with the N-benzylation. This structural identification was further corroborated by the absence of a carbonyl stretch in the IR spectrum. In addition to the N-benzyl product **11,** a sizable amount (27%) of dibenzylated aniline **16** was isolated from this reaction. Treatment of the dibenzyl compound **16** with sodium hydride, diethyl malonate in DMSO at 110°C for 3 days afforded malonate **17** in 55% yield. Malonate **17** was purified by silica gel column chromatography and gave a crystalline product (from ethyl acetate/petroleum ether), which was analyzed by x-ray diffraction. The single x-ray crystal structure of compound **17** given in Figure **3** provided unequivocal support for the unexpected N-benzylation in the reaction of benzyl chloroformate with certain anilines.

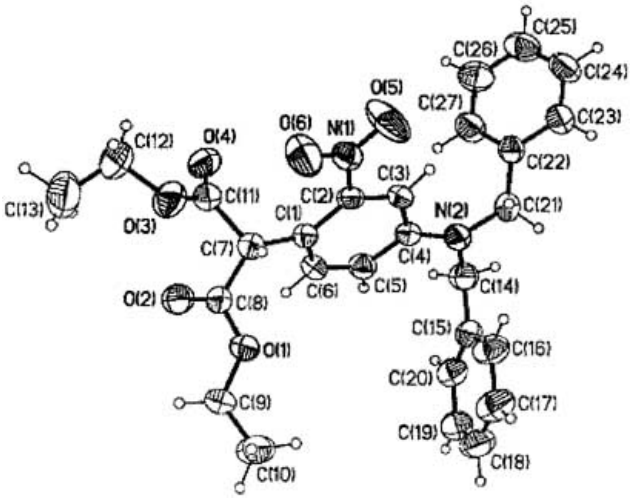


Fig. (3). X-ray crystal structure of compound **17**.

Reaction of the protected aniline **11** with diethyl malonate and sodium hydride in dry DMSO at 110 °C for 64 hours gave the desired diester **12** in 60% yield. Hydrolysis of diester **12** with sodium hydroxide, followed by acidification generated the substituted acetic acid in 95% yield. This carboxylic acid product was selectively reduced with borane/THF at 5°C to give alcohol **13** in 99% yield as a colorless oily residue. This alcohol was converted to the corresponding primary chloride **14** through a reaction with triphenylphosphine and carbon tetrachloride. The orange-colored chloride was produced in 81% yield as a solid. The nitro moiety of chloride **14** was reduced to an amine by hydrogenation at 55 PSI over PtO_2. The amine, which was unstable, was immediately coupled at room temperature to 5,6,7-trimethoxyindole-2-carboxylic acid (TMI) in presence of tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and N,N-diisopropylethylamine. A clear and colorless foamy product **15** was isolated in 33% yield, after column chromatography. The benzyl protecting group was removed by hydrogenation over 10% Pd on carbon in THF at room temperature. The desired achiral *seco*-amino-CI-TMI **1** was isolated as a viscous colorless oil in 78% yield, following purification by silica gel column chromatography. The structures of all intermediates and product **1** were ascertained by analysis of 500 MHz ^{1}H-NMR, FT-IR, EI-MS, FAB-MS, and elemental analysis data.

With target compound **1** available, its ability to inhibit the growth of tumor cells in culture was investigated. Using a MTT based assay [13], exposure of K562 human chronic myeloid leukemia cells to the compound for 4 days gave an IC_{50} value of 1.30 ± 0.3 μM. For comparison, the IC_{50} value for *seco*-CI-TMI **2** was found to be 0.15 ± 0.09 μM. These IC_{50} values agree with the reported cytotoxicity of (+)-*seco*-amino-CI-TMI **5**, which gave an IC_{50} value of 0.13 μM against the growth of EMT6 murine mammary carcinoma, albeit that was done with a 4-hour drug exposure [6]. The cytotoxicity results for compound **1** are intriguing because they demonstrate that the chiral center present in the CC1065 class of compounds is not necessary for the compounds to be biologically active. The results further suggest that the 4-aminophenethyl chloride moiety is a feasible substitute for hydroxy *seco*-analogs of the duocarmycins and CC-1065. Furthermore, compound **1** must be stable in cell culture media and it must have been taken up by the K562 cells in order to exert a reasonable cytotoxic potency, compared to the corresponding chiral amino and hydroxy analogs **5** and **2**, respectively.

To further evaluate the biological properties of the 4-aminophenethyl chloride moiety as a possible substitute for the chiral *seco*-CPI pharmacophore, the ability of compound **1** to covalently react with DNA was investigated. DNA sequencing and thermal-induced cleavage experiments are commonly used for probing the covalent sequence specific reaction of compounds with purine-N3 groups [1,14]. Upon heating, purine-N3 adducts produce specific DNA strand breaks that allow for identification of the exact residue at which the alkylation had occurred. As depicted in Figure **4**, the covalent sequence specificity of compound **1** and adozelesin was assessed by a thermally induced DNA strand cleavage experiment. The DNA fragment used in these

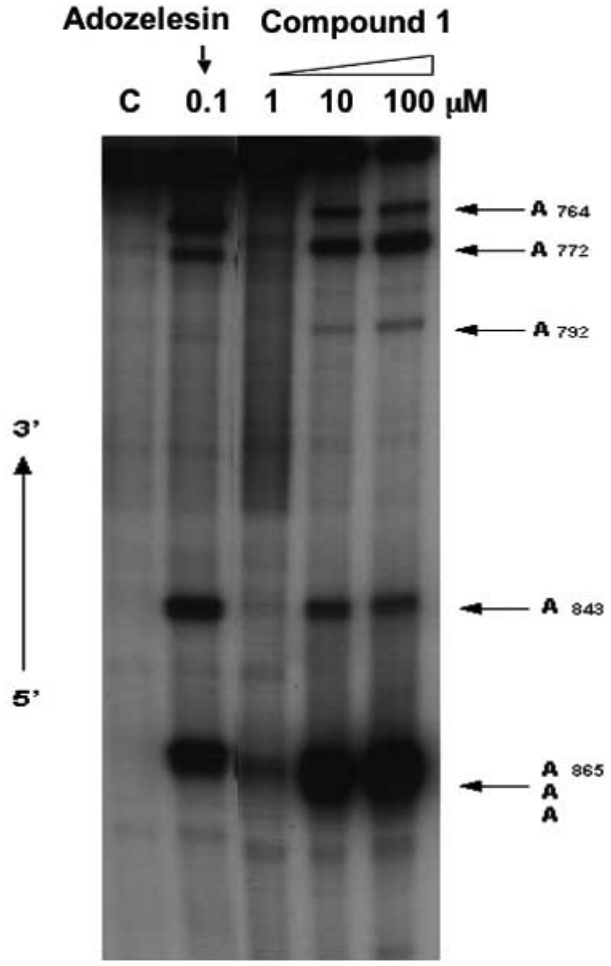


Fig. (4). Thermally induced and sequence selective DNA strand break study on compound **1** and adozelesin.

studies was obtained from PCR amplification of base pairs 745 - 956 of the pUC18 plasmid that was linearized with *Hind III*. A 5'-^{32}P-labeled 5'-TGGTATCTTTATAGTCCT GTCG-3' primer was used as the forward primer so that each final probe copy was singly end-labeled. The results clearly indicate that compound **1** has similar sequence preference to adozelesin and preferentially alkylates the adenine-N3 within 5'-AAAA<u>A</u>-3', consistent with the reported sequence preference of adozelesin and CC- 1065 [1]. This result indicates that the 4-aminophenethyl chloride moiety behaves similarly to the CPI pharmacophore of adozelesin and other CC1065 analogs, further suggesting that the chiral center present in the CPI group is not necessary to exhibiting the sequence specificity observed for this class of compounds.

In conclusion, the results described in this paper provide a systematic study on a novel achiral analog of CC1065 and the duocarmycins. It demonstrates that the 4-aminophenethyl chloride moiety, the first reported achiral analog, is a feasible substitute for the *seco*-CPI pharmacophore, in terms of DNA sequence alkylation and anticancer drug design. Current studies are focused on systematic modification of compound **1** in order to enhance its anticancer potency, and the results from these studies will be reported in due course.

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