Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2693–2699

BIOORGANIC & MEDICINAL CHEMISTRY

Polycyclic Aromatic Compounds as Anticancer Agents: Synthesis and Biological Evaluation of Dibenzofluorene Derivatives

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Received 10 January 2000; accepted 11 July 2000

Abstract—Highly regioselective electrophilic substitution of dibenzofluorene was achieved and the nitro derivative was transformed to a variety of new anticancer agents.

Introduction

The synthesis of polycyclic aromatic ring systems by various methodologies has been published extensively.[1] The carcinogenic properties of such compounds have been explained by advancing a number of different mechanisms.[2] The use of polycyclic aromatic compounds and their derivatives as anticancer agents has also been explored, but less is known regarding this activity.[3] In the main, the antitumor activity of these compounds is proposed to depend on intercalation with or covalent binding to DNA.[4] However, many other sites of interaction such as the cell membrane have been identified. From a prior limited study, we determined that suitably substituted chrysene derivatives act on the cancer cell through interactions with the membrane.[5]

Although using potentially carcinogenic/mutagenic compounds to derive antitumor agents might appear a questionable approach, a large body of information supports this concept. Many reports have demonstrated that alteration of the structure of PAH can mitigate their deleterious effects, emphasizing their interaction with specific cell organelles to evoke specific cytotoxic reactions.[2a] As a result, many of the antitumor agents that are in current clinical use are derived from compounds such as carbazoles, anthracenes, and related structures.[6]

Results and Discussion

Recently, we have developed a novel oxidation method for the conversion of benzylic methylenes to benzylic ketones in polycycylic systems by sodium bismuthate.[7] Thus, pentacyclic dibenzfluorene **1** was oxidized to the dibenzfluorenone **2** in good yield (Scheme 1).

We have also shown a facile reduction of the polycyclic aromatic nitro compounds to polycyclic aromatic amines (for example, **3** to **4**) by samarium metal in the presence of catalytic amounts of iodine (Scheme 2).[8]

Using these two methods, we examined the synthesis and the antitumor activities of structurally complex, *angular* dibenzofluorene [*a,g*] polycyclic systems with a very reactive *bridged* methylene group. We hypothesized that a bridged unit in the polycyclic aromatic system could play an important role in this function as it could form cation, anion, and radical intermediates. This communication describes the electrophilic substitution reaction of the 13*H* dibenzofluorene (**1**) for the first time. The structure–activity relationships of several new diamides and diamines (**13**, **14**, **15** and **16**) are also reported here.

We prepared pentacyclic dibenzo[*a,g*]fluorene (**1**) in 20% yield by following the method reported by Harvey.[9] The hydrocarbon **1** can be prepared via alkylation of the enamine **5a** with the bromide **6** and cyclodehydration–aromatization of the ketone **5b** (Scheme 3).

Functionalization of benzene and naphthalene derivatives by electrophilic reaction[1a,10] is routine organic

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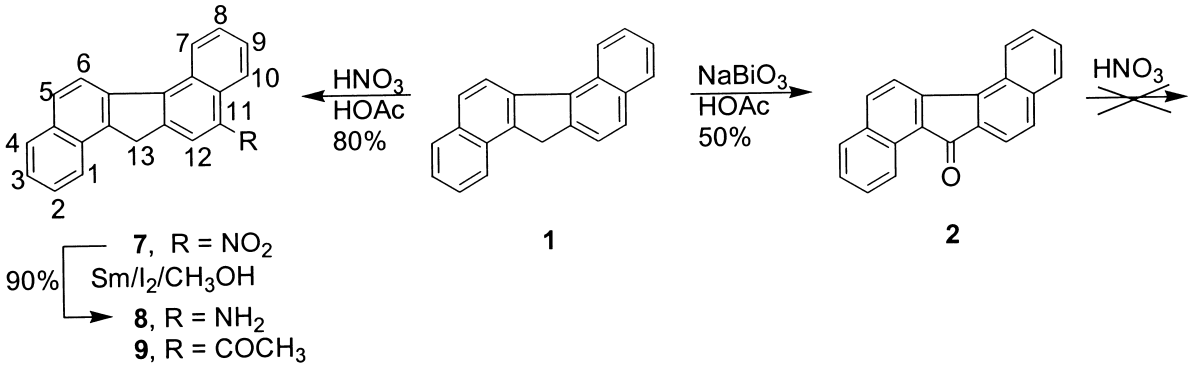
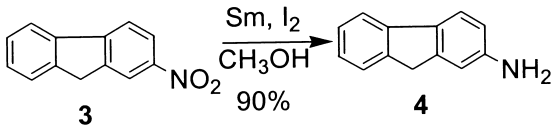
0968-0896/00/$ - see front matter
PII: S0968-0896(00)00213-3



Scheme 1.



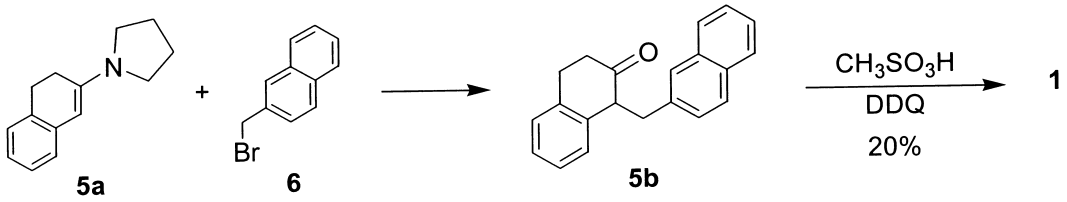
Scheme 2.

chemistry. The orientation of the electrophile in such monocyclic or bicyclic derivatives is predictable. A similar substitution reaction in a polycyclic aromatic system is extremely difficult, and for polycyclic non-alternate hydrocarbon it is poorly predictable. In fact, little is known about the electrophilic substitution reaction in polycyclic aromatic system.[11]

We planned to link a 4-carbon side chain with a heterocyclic base at the end to the aromatic ring through nitrogen. Therefore, our task was to prepare amino dibenzofluorene **8** for the subsequent derivatization. Towards this goal, we reacted the ketone **2** with nitric acid in acetic acid under different conditions but failed to produce the desired nitro derivative. However, the hydrocarbon **1** produced a single nitro compound **7** with nitric acid–acetic acid at 0–5 °C in 80% yield. The location of the nitro group in the aromatic system was determined by NMR spectra. The NMR spectrum (400 MHz) of the known hydrocarbon **1** was taken. Based on the homonuclear decoupling and COSY NMR studies, all of the protons in **1** were assigned as follows: (400 MHz, $CDCl_3$) δ 8.81 (1H, d, $J = 8.46$ Hz, H_7), 8.50 (1H, d, $J = 8.68$ Hz, H_6), 8.02 (1H, d, $J = 8.22$ Hz, H_1), 7.89–7.96 (3H, m, H_4, H_5, H_{10}), 7.70 and 7.79 (2H, ABq, $J = 8.23$ Hz, H_{11}, H_{12}), 7.60–7.65 (1H, m, H_8), 7.45–7.54 (3H, m, H_2, H_3, H_9). These assignments were supported by the data reported by Jones et al.[12] The NMR spectrum revealed that the AB quartet present in **1** was absent from the nitro compound **7**. The spectrum of **7** showed a new singlet at δ 8.39 and a new doublet at δ 8.44 ($J = 8.75$ Hz). We eliminated positions C_1, C_4, C_7 and C_{10} for the nitro group because of the singlet at δ 8.44. The positions C_5, C_6, C_8 and C_9 were eliminated based on the homonuclear decoupling and COSY experiment. That region (δ 7.5–7.6) of the hydrocarbon **1** remained unaffected in **7** clearly ruled out positions C_2 and C_3 for the nitro group in **7**. We eliminated position C_{12} because of the downfield doublet at δ 8.44. The ^{13}C NMR spectrum of **7** showed the presence of nine quaternary carbons. The signal at δ 123.58 due to the C_{11} carbon (verified by HETCOR study) was not present in the spectrum of **7**. A new peak at δ 145.24 appeared because of the nitro group. Thus, we assigned C_{11} as the site of the nitro group in **7** based on extensive NMR study.

The site of electrophilic attack was further verified by computer-assisted charge density calculation of the Whelands intermediates.[13] The relative energies of the Wheland intermediates of each of the carbons were calculated as C_1 (6.947), C_2 (21.278), C_3 (13.68), C_4 (11.008), C_5 (14.403), C_6 (16.919), C_7 (0.0), C_8 (10.558), C_9 (8.591), C_{10} (0.894), C_{11} (2.325), C_{12} (19.638), and C_{13} (7.358). These values indicated that preferential attack can take place at positions 7, 10, and 11. On the basis of NMR data we eliminated positions 7 and 10.

In order to study the electrophilic substitution reaction of the hydrocarbon **1**, we carried out acetylation by acetic anhydride in the presence of anhydrous aluminum chloride using dichloromethane as solvent. The crude NMR showed the presence of one acetyl group. The location of the acetyl group in **9** was tentatively assigned based on the location of the nitro group in the aromatic system (Scheme 1). Regioselective electrophilic substitution



Scheme 3.

of **1** is interesting and the use of **9** will be described in subsequent publications.

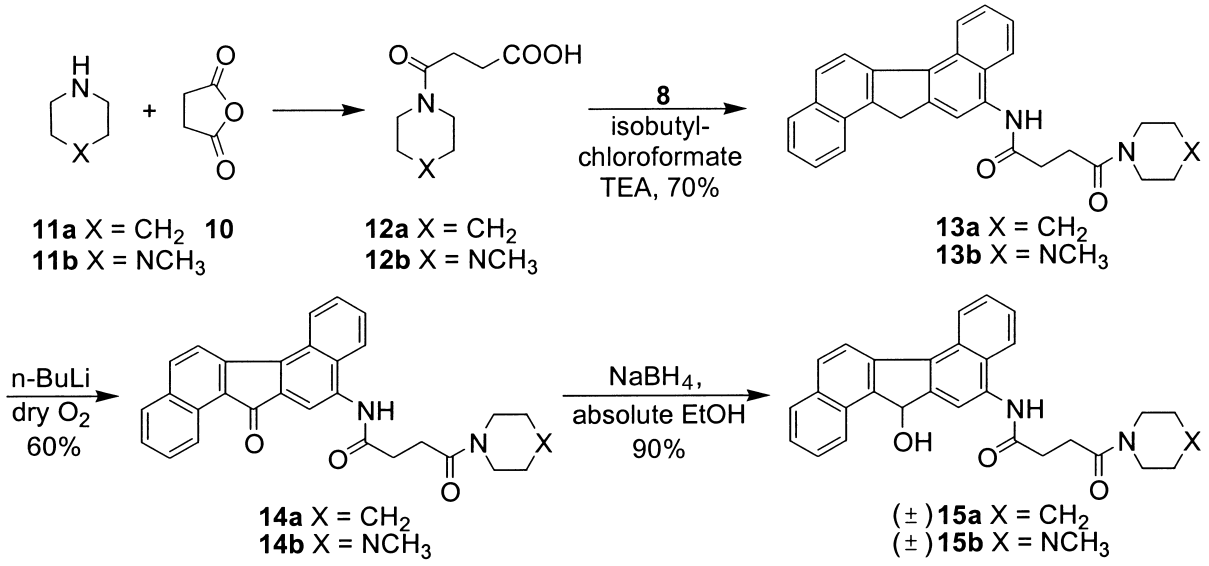
Reduction of the nitro compound **7** to the amino compound **8** was carried out by following our own methodology.[8] We demonstrated that samarium–iodine is highly effective for the transformation of nitro to the amino compound. Our next task was to prepare the side chains **12** and to couple them to the amine **8**. The acid **12** was prepared by refluxing succinic anhydride (**10**) with piperidine (**11a**) and *N*-methylpiperazine (**11b**). The amine **8** was then condensed with the side chains **12** by mixed anhydride method. Many other condensing agents, such as DCC[14] and HOBt, failed to give the amides **13** (Scheme 4).

The desired diamides **13a** and **13b** were isolated by column chromatography. The benzylic methylene group in **13** was oxidized by molecular oxygen[15] to get the ketones **14a** and **14b**. The oxidation of **13** that has a bulky side chain is noteworthy since it might be expected to interfere with the reaction. The ketone **14** was subsequently reduced to the alcohol **15**.

Reduction of the amide functionality to the amino group by lithium aluminum hydride is a standard chemical transformation. Therefore, in order to produce the diamino compounds, reduction of **13** was carried out by this reagent under a variety of conditions. However, neither of the desired diamino compounds **16a** or **16b** could be isolated from the complex reaction mixtures by this method. After many experiments, diborane was found to be the reagent of choice for this reaction and it resulted in a good yield of the amino compound **16**.

These derivatives were tested in our Center's Core Analytical Laboratory against three tumor lines of animal origin and six of human origin, all of which have been used in the NCI panel for the testing of chemotherapeutic agents. As an approach to an operational definition of in vitro, antitumor cytotoxicity (activity), we chose to compare the effects of our compounds with those of cisplatin.

The antitumor activity of these newly synthesized dibenzofluorene derivatives **13**, **14**, **15** and **16** were tested and a comparison with respect to cisplatin shown in Table 1. The results of these tests are of interest both in terms of their antitumor effects and in the significant alterations in activity that occurred with minor structural modifications. Compound **13b** was more active than cisplatin against four of the five tumors against which cisplatin

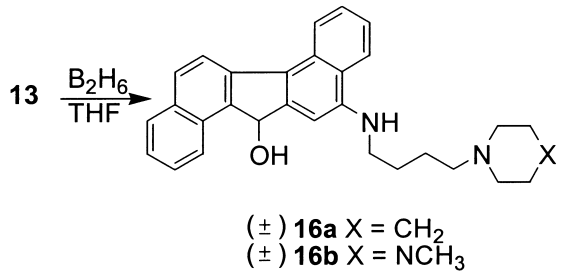


Scheme 4.

Table 1. IC_{50} (μM) of compounds **13** to **15** MTT assay (72 h continuous exposure)[a]

Cell line	Cisplatin	13a	13b	14a	14b	15a	15b	16a	16b
B16	7.33	128.63	4.31	121.93	12.35	29.70	11.05	4.04	1.61
BRO	5.66	103.44	3.88	120.64	12.77	31.64	12.51	4.28	3.64
HL-60	—	33.22	3.45	74.15	7.33	12.05	4.17	—	–
L1210	—	53.50	3.88	51.24	2.93	17.44	6.05	—	–
MCF-7	15.99	50.83	4.31	78.26	25.75	19.16	12.30	4.75	4.36
OVCAR 3	—	88.06	4.09	97.94	13.82	18.94	8.76	3.09	1.84
P388/0	—	95.64	4.31	81.72	2.72	19.80	4.38	4.51	4.36
PC 3	1.66	33.44	3.88	31.35	27.22	13.99	6.46	4.04	3.44
HT-29	15.99	> 100	3.88	61.40	21.78	10.08	13.55	4.04	3.21

[a]All data were provided as IC_{50} values (μM) and assays were conducted by 72 h continuous exposure using the MTT method. The final concentration of solvent was < 0.625% which was not toxic to the cells. All dilutions were made in RPMI 1640 with 10% FBS. The cytotoxity data is based on at least three separate experiments with deviations within 0.2 μM.



Scheme 5.

was used. This places it as one of the most effective compounds we have yet derived from a polycyclic hydrocarbon. The addition of a ketone group at position 13 created the fluorenone compound **14b** and resulted in a decrease of activity against all but the animal leukemic lines L1210 and P388. The presence of a piperidine heterocyclic group at the terminus of the alkyl chain appears to render these compounds relatively inactive when compared with those that terminate with the heterocyclic *N*-methyl piperazine group. Compounds **13a** versus **13b** and **14a** versus **14b** are striking examples of this drastic alteration in activity and appear to represent an off/on phenomenon. While addition of a hydroxyl group at position 13 in compound **15b** produced either a reduction or no significant alteration in activity when compared with its parent compound **13b**, this addition in **15a** significantly increased activity when compared with **13a**.

Previous work[5] in our laboratory with chrysene as the PAH nucleus demonstrated that reduction of the diamides significantly increased antitumor activity, especially for the relatively inactive compounds. Reduction of **13a** and **13b** resulted in their diamine derivatives **16a** and **16b**, respectively. An impressive increase was seen when **13b** was compared to **16b** against B16 and OVCAR; while in all other instances, **16b** were equally active.

Even more striking was the enhancement of activity when the diamine **16a** was compared with its diamide analogue **13a**. While IC_{50} of the latter was greater than 30 μM against every cell line, **16a** demonstrated IC_{50} of less than 5 μM against seven out of seven tumor lines. The cellular basis for the remarkable differences of activity of the two series of compounds in all cell lines has not been identified.

Conclusion

In conclusion, we have demonstrated for the first time electrophilic substitution reaction of 13*H*-dibenzo[*a*,*g*]-fluorene (**1**). The prominent antitumor activity of some of the new[16] compounds **13**, **14**, **15** and **16** opens up an approach to research at the chemical:biologic interface. That the agents described herein are producing certain of their in vitro effects against human and animal cancer lines through a specific cytotoxicity is exemplified by the finding, against nine tumor lines, that **14b** had an IC_{50} less than 5 μM against two, less than 10 μM against one, between 10 and 20 μM against three, and above 20 μM against three. In a similar manner **15b** had an IC_{50} less than 5 μM against two lines, less than 10 μM against three, and above 10 μM against four.

Experimental

General methods

All reactions described in this paper were carried out under a well-ventilated hood. CH_2Cl_2 and THF were dried and freshly distilled in the usual way before use. IR spectra were recorded on a Perkin–Elmer instrument and UV spectra were recorded on a Perkin–Elmer instrument in nm. NMR spectra were recorded on Bruker 200 MHz and 300 MHz spectrometers. Chemical shifts were reported as δ values in parts per million downfield from tetramethyl silane as the internal standard in $CDCl_3$. Mass spectra were obtained on a Micromass VG platform with a single quadrapole and fitted with an electrospray source. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Inc., New York. Melting points were taken in open capillary tube and are not corrected. Column chromatography was carried out with Aldrich silica gel (230 mesh). TLC was run with precoated silica gel plate. Na_2SO_4 was used as the drying agent after all the extractions.

11-Nitro-13*H*-dibenzofluorene (7). To an ice-cold solution of the hydrocarbon **1** (900 mg, 3.38 mmol) in THF (30 mL) was added glacial acetic acid (50 mL). Nitric acid (90%, 14 mL) was added dropwise to the stirred solution of the hydrocarbon **1** at 0–5 °C. After the addition was complete, stirring was continued for 2 h (0 °C–RT). The reaction mixture was poured into crushed ice, filtered, and washed with water until free from acid. The crude product was crystallized from CH_2Cl_2–hexanes to afford **7** (80% yield).

11-Amino-13*H*-dibenzofluorene (8). Nitro compound **7** (0.80 mmol) was reduced by samarium metal (3 mmol) in the presence of iodine as described in our previous publication[7] to afford the amine **8** as a dark semi-solid mass; yield 90%; UV: λ_{max} 231.96 (log ε = 2.26), λ_{max} 264.26 (log ε = 2.76), λ_{max} 366.09 (log ε = 4.33); IR (neat) 3422, 3346, 3050, 2954, 2917, 2849, 1699, 1621, 1587, 1515, 1456 cm^{-1}; ^1H NMR (200 MHz) δ 8.84–8.82 (1H, d), 8.47–8.44 (1H, d), 8.04–7.80 (3H, m), 7.69–7.43 (7H, m), 7.16 (1H, s), 4.18 (2H, s); ^{13}C NMR (400 MHz) 143.60, 141.55, 140.80, 139.05, 131.10, 130.45, 130.14, 128.89, 128.64, 127.55, 126.45, 126.28, 124.62, 124.28, 124.07, 123.76, 123.30, 121.89, 120.95, 107.78, 36.48; Mass: 282.04 $(M+H)^+$.

11-Acetyl-13*H*-dibenzofluorene (9). To an ice-cold solution of the hydrocarbon **1** (900 mg, 3.38 mmol) in dry CH_2Cl_2 (50 mL) was added acetic anhydride (460 mg, 4.5 mmol) followed by anhydrous aluminum chloride (800 mg, 6.0 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was decomposed with dilute hydrochloric acid and was extracted with CH_2Cl_2, washed with brine, dried, and

evaporated. The crude product was crystallized from CH_2Cl_2–hexanes to afford **9** (90% yield), mp 141 °C, IR(CH_2Cl_2) 1667 cm^{-1}; ^1H NMR (200 MHz) δ 9.08–8.76 (2H, m, Ar), 8.45 (1H, d, J = 8.78 Hz, Ar), 8.10 (1H, s, H_{12}), 8.02 (1H, d, J = 7.82 Hz, Ar), 7.91 (2H, d, J = 7.83 Hz, Ar), 7.74–7.42 (4H, m, Ar), 4.16 (2H, s, benzylic CH_2), 2.80 (3H, s, $COCH_3$).

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorenyl)]-4-(1′-piperidinyl)-butane-1,4-dicarboxiamide (13a).** To an ice cold solution of the acid **12a** (1.065 g, 5.76 mmol) in dry CH_2Cl_2 (50 mL) was added dry triethylamine (801 μL, 5.76 mmol) followed by freshly distilled isobutyl chloroformate (664 μL, 5.12 mmol). Stirring was continued in cold conditions for 5 min. Next this mixed anhydride was added dropwise to an ice cold suspension of the 11-amino 13*H*-dibenzofluorene (**8**) (719 mg, 2.56 mmol) in dry CH_2Cl_2 (50 mL), and stirring was continued overnight, after which TLC in ethyl acetate:hexanes (20%) showed the absence of the starting amine. The CH_2Cl_2 layer was washed successively with HCl (5%), brine, $NaHCO_3$ (5%), and brine and then dried. Removal of the solvent under vacuo afforded the crude product, which was purified by column chromatography over silica gel and elution with ethyl acetate to furnish **13a** (800 mg, 70%); mp 186–188 °C; UV (CH_2Cl_2) λ_{max} 259.41 (log ε = 4.67), λ_{max} 358.33 (log ε = 4.43); IR (neat) 3264, 2934, 1648, 1538, 1509, 1442, 1259, 804, 741 cm^{-1}; ^1H NMR (200 MHz) δ 9.39 (1H, s, ArNHCO-), 8.86 (J = 8.40 Hz, 1H, d, H_7), 8.47 (J = 8.70 Hz, 1H, d, H_6), 8.37 (1H, s, H_{12}), 8.16 (J = 8.31 Hz, 1H, d, H_{10}), 8.05 (J = 8.03 Hz, 1H, d, H_1), 7.91 (J = 9.29 Hz, 2H, d, Ar), 7.66–7.45 (4H, m, Ar), 4.24 (2H, s, benzylic CH_2), 3.78–3.58 (2H, brt, -$CONCH_2$), 3.56–3.38 (2H, brt,-$CONCH_2$), 3.05–2.75 (4H, m, -$COCH_2CH_2CO$-), 1.82–1.48 (6H, brs, -$NCH_2(CH_2)_3CH_2$-); ^{13}C NMR (400 MHz): 170.929, 169.439, 141.249, 139.962, 138.970, 133.079, 130.982, 130.609, 129.398, 128.808, 127.584, 126.607, 125.620, 125.392, 125.169, 124.231, 124.089, 123.156, 123.076, 121.385, 120.342, 116.504, 45.524, 42.215, 35.613, 32.088, 28.705, 25.325, 24.618, 23.442; Mass (ES^+): 449, 279, 205, 167, 149, 145. Anal. calcd for $C_{30}H_{28}N_2O_2$: C, 80.30; H, 6.30; N, 6.20%. Found: C, 80.36; H, 6.02; N, 6.27%.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorenyl)]-4-(4′*N*-methyl-piperazinyl)-butane-1,4-dicarboxiamide (13b).** To an ice-cold solution of the acid **12b** (1.188 g, 5.94 mmol) in dry CH_2Cl_2 (100 mL) was added dry triethylamine (826 μL, 5.94 mmol) followed by freshly distilled isobutyl chloroformate (686 μL, 5.29 mmol) and stirring was continued in cold conditions for 5 min. Next, this mixed anhydride was added dropwise to an ice cold suspension of the 11-amino 13*H*-dibenzofluorene (**8**) (742 mg, 2.64 mmol) in dry CH_2Cl_2 (60 mL), and stirring was continued overnight; after that, the TLC in ethyl acetate:hexanes (20%) showed the absence of the starting amine. The CH_2Cl_2 layer was washed successively with $NaHCO_3$ (5%) and brine and then dried. Removal of the solvent under vacuo afforded the crude product, which was purified by column chromatography over silica gel and elution with MeOH to furnish **13b** (800 mg, 65%); mp 200–202 °C; UV (CH_2Cl_2) λ_{max} 259.42 (log ε = 4.35), λ_{max} 358.28 (log ε = 4.016); IR (neat). 3262, 2920, 1643, 1538, 1442, 1292, 1259, 1143, 803, 740 cm^{-1}. ^1H NMR (200 MHz) δ 9.15 (1H, s, ArNHCO-), 8.85 (J = 8.40 Hz, 1H, d, H_7), 8.45 (J = 8.70 Hz, 1H, d, H_6), 8.30 (1H, s, H_{12}), 8.12 (J = 8.32 Hz, 1H, d, H_{10}), 8.05 (J = 8.03 Hz, 1H, d, H_1), 7.91 (J = 8.00 Hz, 2H, d, Ar), 7.74–7.34 (4H, m, Ar), 4.20 (2H, s, benzylic CH_2), 3.84–3.66 (2H, brt, -$CONCH_2$), 3.62–3.42 (2H, brt, -$CONCH_2$), 3.05–2.75 (4H, m,-$COCH_2CH_2CO$-), 2.52–2.18 (7H, m, -$(CH_2)_2NCH_3$, with a singlet at δ 2.30 for -NCH_3); ^{13}C NMR (300 MHz) δ 171.692, 170.628, 142.137, 140.963, 139.871, 134.221, 131.718, 131.569, 130.334, 129.762, 128.563, 127.604, 126.633, 126.388, 126.192, 125.248, 125.117, 124.179, 124.055, 122.261, 121.314, 117.702, 54.8863, 54.6109, 45.9947, 45.2696, 41.9344, 36.5679, 32.8116, 29.5007; Mass (ES^+): 464, 257, 191, 146, 145. Anal. calcd for $C_{30}H_{29}N_3O_2$: C, 77.70; H, 6.30; N, 9.10%. Found: C, 77.46; H, 6.01; N, 8.46%.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorene-13′-one]-4-(1′-piperidinyl)-butane-1,4-dicarboxiamide (14a).** To a solution of **13a** (665 mg, 1.48 mmol) in dry THF (40 mL) at −78 °C under argon was added a solution of *n*-BuLi (1.8 mL, 2.5 M, 4.44 mmol) in cyclohexane. The deep-yellow colored solution was stirred for 1 h, 30 min at this temperature, and then dry O_2 was bubbled through the solution for 1 h. The temperature was allowed to rise to room temperature while O_2 continued to bubble through the solution for an additional 3 h. The reaction was quenched by the addition of water (20 mL) and CH_2Cl_2 (30 mL), and stirring was continued for 30 min. The organic layer was collected, washed with brine, and dried. On evaporation of the solvent under vacuo, the crude product was obtained, which was crystallized from CH_2Cl_2/hexanes to yield **14a** (452 mg, 66%); mp 226–228 °C; UV (CH_2Cl_2), λ_{max} 229.60 (log ε = 4.22), λ_{max} 285.05 (log ε = 4.28), λ_{max} 331.39 (log ε = 4.05); IR (neat) 3244, 2938, 1698, 1644, 1518, 1445, 1265, 815, 801, 735, 703 cm^{-1}; ^1H NMR (200 MHz) δ 9.43 (1H, s, ArNHCO-), 8.94 (J = 8.44 Hz, 1H, d, Ar), 8.52–8.48 (1H, m, Ar), 8.24–8.00 (3H, m, Ar), 7.90 (J = 8.45 Hz, 1H, d, Ar), 7.70 (J = 8.16 Hz, 1H, d, Ar), 7.65–7.48 (3H, m, Ar), 7.39 (J = 8.06 Hz, 1H, d, Ar), 3.82–3.64 (2H, brt, -$CONCH_2$), 3.62–3.38 (2H, brt, -$CONCH_2$), 2.87 (4H, s,-$COCH_2CH_2CO$-), 1.80–1.50 (6H, brs, -$NCH_2(CH_2)_3CH_2$-); ^{13}C NMR (300 MHz, $CDCl_3$) δ 195.748, 171.875, 170.471, 147.007, 137.940, 135.158, 134.817, 133.708, 132.037, 130.713, 130.103, 129.348, 129.263, 128.138, 127.830, 127.487, 126.996, 126.236, 124.990, 124.336, 123.285, 120.887, 114.342, 46.562, 43.236, 26.331, 25.625, 24.419, 22.662, 14.125; Mass (ES^+): 463, 186, 152, 147, 145. Anal. calcd for $C_{30}H_{26}N_2O_3$: C, 77.90; H, 5.70; N, 6.10%. Found: C, 77.69; H, 5.64; N, 5.99%.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorene-13′-one]-4-(4′*N*-methyl-piperazinyl)-butane-1,4-dicarboxiamide (14b).** To a solution of **13b** (500 mg, 1.08 mmol), in dry THF (50 mL) at −78 °C under argon was added a solution of *n*-BuLi (1.30 mL, 2.5 M, 3.24 mmol) in cyclohexane. The deep-yellow colored solution was stirred for 1 h, 30 min at this temperature, and then dry O_2 was bubbled through the solution for 1 h. The temperature was allowed to rise

to room temperature while O_2 continued to bubble through the solution for an additional 3 h. The reaction was quenched by the addition of water (20 mL) and CH_2Cl_2 (50 mL), and stirring was continued for 30 min. The organic layer was collected, washed with brine, and dried. On evaporation of the solvent under vacuo, the crude product was crystallized from CH_2Cl_2–hexanes to furnish **14b** (350 mg, 68%); mp 204–206 °C; UV (CH_2Cl_2), λ_{max} 228.88 (log ε = 4.29), λ_{max} 285.58 (log ε = 4.30), λ_{max} 330.50 (log ε = 4.06); IR (neat) 3231, 2920, 2852, 1699, 1645, 1540, 1519, 1459, 1276, 1262, 1004, 815, 751, 701 cm^{-1}; ^1H NMR (200 MHz) δ 9.17 (1H, s, ArNHCO-), 8.93 (J = 8.57 Hz, 1H, d, Ar), 8.48–8.35 (1H, m, Ar), 8.30–7.96 (3H, m, Ar), 7.86 (J = 8.54 Hz, 1H, d, Ar), 7.69 (J = 8.33 Hz, 1H, d, Ar), 7.60–7.42 (3H, m, Ar), 7.38 (J = 7.82 Hz, 1H, d, Ar), 3.86–3.40 (4H, m, CON $(CH_2)_2$), 2.97 (4H, s, -$COCH_2CH_2CO$-), 2.62–2.16 (7H, m, (-$CH_2)_2$ NCH_3 with a singlet at δ 2.31 for-NCH_3); ^{13}C NMR (300 MHz) δ 195.507, 171.684, 170.674, 146.779, 138.031, 135.070, 134.472, 133.633, 131.810, 130.830, 129.982, 129.267, 129.123, 128.098, 127.749, 127.446, 126.858, 126.163, 124.891, 124.270, 123.225, 120.801, 114.643, 54.920, 54.643, 46.003, 45.332, 31.590, 22.657, 14.123; Mass (ES^+): 478, 464, 147, 145. Anal. calcd for $C_{30}H_{27}N_3O_3$: C, 75.50; H, 5.70; N, 8.80%. Found: C, 75.19; H, 5.84; N, 7.99%.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorene-13′-hydroxy]-4-(1′-piperidinyl)-butane-1,4-dicarboxiamide (15a).** To a solution of **14a** (200 mg, 0.43 mmol) in absolute EtOH (20 mL) was added $NaBH_4$ (47 mg, 1.29 mmol) at 0–5 °C and the solution was stirred for 30 min. The stirring was continued for an additional 4 h at room temperature. The reaction was quenched by the careful addition of water (10 mL). The mixture was extracted with CH_2Cl_2 (2×20 mL), and the organic layer was washed with brine and dried. On removal of the solvent under vacuo, the crude product was crystallized from CH_2Cl_2–hexanes to yield **15a** (150 mg, 75%); mp 220–222 °C; UV (CH_2Cl_2), λ_{max} 228.00 (log ε = 4.66), λ_{max} 261.79 (log ε = 4.89), λ_{max} 366.69 (log ε = 4.42); IR (neat) 3263, 2935, 2855, 1647, 1624, 1538, 1443, 1276, 1262, 1183, 1088, 808, 748, 684 cm^{-1}; ^1H NMR (200 MHz) δ 9.26 (1H, s, ArNHCO-), 8.72 (J = 8.62 Hz, 1H, d, Ar), 8.41 (J = 8.07 Hz, 1H, d, Ar), 8.20–8.12 (2H, m, Ar with a singlet at 8.28 for H_{12}), 8.00–7.65 (3H, m, Ar), 7.60–7.28 (4H, m, Ar), 5.83 (J = 10.28 Hz, 1H, d, H_{13}), 3.75–3.55 (2H, brt, -$CONCH_2$), 3.50–3.35 (2H, brt,-$CONCH_2$), 3.00–2.62 [5H, m, -$COCH_2CH_2CO$- with a doublet at 2.70 (J = 10.28 Hz, C_{13}-OH, which disappeared with D_2O)], 1.78–1.44 [6H, brs, -NCH_2 $(CH_2)_3$ CH_2-]; Mass (ES^+): 465, 447, 168. Anal. calcd for $C_{30}H_{28}N_2O_3$: C, 77.90; H, 5.70; N, 6.10%. Found: C, 77.92; H, 5.92; N, 5.63%.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorene-13′-hydroxy]-4-(4′*N*-methyl-piperazinyl)-butane-1,4-dicarboxiamide (15b).** To a solution of **14b** (200 mg, 0.42 mmol) in absolute EtOH (20 mL) was added $NaBH_4$ (46 mg, 1.26 mmol) at 0–5 °C, and the solution was stirred under this condition for 30 min. The temperature was allowed to rise to room temperature, and stirring was continued for an additional 4 h. The reaction was quenched by the careful addition of water (10 mL) and the reactants were extracted with CH_2Cl_2 (50 mL), washed with brine, and dried. On removal of the solvent under vacuo, crude product was crystallized from CH_2Cl_2/hexanes to yield 140 mg (70%) of pure **15b**; mp 166–168 °C; UV (CH_2Cl_2) λ_{max} 227.17 (log ε = 4.46), λ_{max} 261.71 (log ε = 4.66), λ_{max} 366.17 (log ε = 4.18); IR (neat) 3267, 2803, 1624, 1539, 1508, 1445, 1276, 1290, 1258, 1183, 1146, 1001, 812, 748, 686 cm^{-1}; ^1H NMR (200 MHz) δ 9.06 (1H, s, ArNHCO-), 8.66 (J = 8.48 Hz, 1H, d, Ar), 8.44 (J = 8.26 Hz, 1H, d, Ar), 8.26 (J = 8.14 Hz, 1H, d, Ar), 8.14 (1H, s, H_{12}), 8.04–7.70 (3H, m, Ar), 7.66–7.32 (4H, m, Ar), 5.82 (1H, s, H_{13}), 3.70–3.12 (4H, m, -$CON(CH_2)_2$-), 2.84–2.50 (4H, m,-$COCH_2CH_2CO$-), 2.35–2.06 (7H, m, (-$CH_2)_2NCH_3$, with a singlet at 2.09 for -NCH_3); Mass (ES^+): 480, 464, 147, 145. Anal. calcd for $C_{30}H_{29}N_3O_3$: C, 75.10; H, 6.10; N, 8.80%. Found: C, 75.59; H, 6.19; N, 8.08%.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorenyl]-4-(1′-piperidinyl)-butane-1,4-diamine (16a).** To a solution of **13a** (50 mg, 0.12 mmol) in THF (50 mL) were added borane–methyl sulphide complex (67 μL, 5 M) solution in diethyl ether under argon at 0 °C, and the mixture was refluxed for 16 h. Then, HCl (15 mL, 5%) was added, and the mixture was refluxed for another 10 h. The solution was cooled, added to 1 M sodium hydroxide solution, extracted with ethyl acetate, washed with brine, and dried. On removal of the solvent, the crude diamine solidified, which was crystallized from ethyl acetate: hexanes (20:80) to give the desired diamine **16a** (35 mg, 75%); mp 166–168 °C; UV (CH_2Cl_2), λ_{max} 229.42 (log ε = 4.50), λ_{max} 268.38 (log ε = 4.55), λ_{max} 377.24 (log ε = 4.25); IR (neat) 3383, 2932, 2767, 1619, 1587, 1570, 1532, 1473, 1443, 1345, 1289, 1182, 1122, 1040, 804, 766 and 683 cm^{-1}; ^1H NMR (200 MHz) δ 8.81 (J = 8.11 Hz, 1H, d), 8.44 (J = 8.70 Hz, 1H, d), 8.14–7.90 (4H, m, Ar), 7.76–7.32 (4H, m, Ar), 6.97 (1H, s, H_{12}), 4.24 (2H, s, benzylic CH_2), 3.40 (J = 6.56 Hz, 2H, t, $ArNHCH_2$-), 2.60–2.24 [6H, brt, H_2C-$N(CH_2)_2$-], 1.98–1.42 (10H, m, methylenes); ^{13}C NMR (300 MHz) δ 144.274, 143.297, 141.144, 138.722, 130.925, 130.487, 130.205, 128.641, 127.459, 126.725, 126.276, 126.195, 124.353, 124.292, 123.758, 123.712, 123.049, 121.090, 120.871, 102.292, 58.903, 54.602, 44.259, 36.837, 27.324, 25.807, 24.818, 24.388; Mass (ES^+): 421, 281, 252, 232, 211, 108.

***N*-[2′-(13′*H*-Dibenzo[*a*,*g*]-fluorenyl)]-4-(4′*N*-methyl-piperazinyl)-butane-1,4-diamine (16b).** To a solution of **13b** (100 mg, 0.22 mmol) in THF (60 mL) was added borane–methyl sulphide complex (130 μL), 5 M solution in diethyl ether under argon at 0 °C, and the mixture was refluxed for 16 h. Then, distilled water (15 mL) was added and the refluxing was continued for an additional 10 h. The solution was cooled, added to 1 M sodium hydroxide solution, extracted with ethyl acetate, washed with brine, and dried. On removal of the solvent, the crude diamine solidified, which was crystallized from ethyl acetate:hexanes (20:80) to give the desired diamine **16b** (62 mg, 66%); mp 140–142 °C; UV (CH_2Cl_2), λ_{max} 227.48 (log ε = 4.43), λ_{max} 268.32 (log ε = 4.45), λ_{max} 377.26 (log ε = 4.14); IR (neat) 376, 2936, 2802, 1619, 1587, 1570, 1514, 1415, 1344, 1241, 1165, 1012, 806, 741,

and 684 cm^{-1}; ^1H NMR (200 MHz) δ 8.80 ($J = 8.35$ Hz, 1H, d), 8.42 ($J = 8.70$ Hz, 1H, d), 8.14–7.88 (4H, m, Ar), 7.72–7.30 (4H, m, Ar), 6.95 (1H, s, H_{12}), 4.22 (2H, s, benzylic CH_2), 3.38 ($J = 6.62$ Hz, 2H, t, $ArNHCH_2$-), 2.68–2.30 [10H, brt, (5X-NCH_2)], 2.30 (3H, s, NCH_3), 1.98–1.56 (4H, m, methylenes); ^{13}C NMR (300 MHz) δ 144.251, 143.231, 141.112, 138.728, 130.938, 130.482, 130.202, 128.645, 127.474, 126.789, 126.285, 126.209, 124.345, 124.322, 123.784, 123.705, 123.035, 121.045, 120.865, 102.332, 58.0515, 54.9487, 53.0379, 45.9162, 44.2362, 40.9995, 36.8472, 29.7030, 27.2063, 24.8132; Mass (ES^+): 436, 281, 260, 239, 219.

Acknowledgements

We gratefully acknowledge the funding support received for this research project from the Hubert L. and Olive Stringer Chair for the partial support of this research. We are thankful to NIH Cancer Center Support Grant, 5-P30-CA16672-25, in particular the shared resources of the Pharmacology and Analytical Center Facility. The antitumor activity was assayed by Dr. Robert Newman in the Pharmacology and Analytical Core Laboratory (2P30CA16672-23).

References and Notes

1. For example, see: (a) Harvey, R. G. *Polycyclic Aromatic Hydrocarbons*; Wiley-VCH, 1997. (b) Clar, E. *Polycyclic Hydrocarbons*; Academic: New York, 1964.
2. (a) Zhang, F.-J.; Cortez, C.; Harvey, R. G. *J. Org. Chem.* **2000**, *65*, 3952. (b) Harvey, R. G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*; Cambridge University Press, 1991; Chapter 4. (c) Di Raddo, P.; Chan, T. *J. Org. Chem.* **1982**, *47*, 1427. (d) Lehr, R. E.; Yagi, H.; Thakker, D. R.; Levin, W.; Conney, A. H.; Jerina, D. M. *Carcinog.-Comprv.* **1978**, *3*, 231. (e) Thakker, D. R.; Yagi, H.; Lu, A. Y. H.; Levin, W.; Conney, A. H.; Jerina, D. M. *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 3381.
3. Smalley, R. V.; Goldstein, D.; Bullowski, D.; Hannon, C.; Buchler, D.; Knudsen, C.; Tuttle, R. L. *Invesi. New Drugs* **1992**, *10*, 107.
4. For some recent references, see: (a) Sami, S. M.; Dorr, R. T.; Alberts, D. S.; Remers, W. A. *J. Med. Chem.* **1993**, *36*, 765. (b) Motohashi, N.; Gollapudi, S. R.; Emrani, J.; Bhattiprolu, K. R. *Cancer Invest.* **1991**, *9*, 305. (c) Denny, W. A.; Rewcastle, G. W.; Baguley, B. C. *J. Med. Chem.* **1990**, *33*, 814. (d) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, *31*, 707.
5. Becker, F. F.; Banik, B. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2877. For an example, see: Jorgensen, K.; Ipsen, J. H. *Biochem. Biophys. Acta* **1991**, *1062*, 227.
6. For some recent examples, see: (a) Cherubim, P.; Deady, L. W.; Dorkos, M.; Quazi, N. H.; Baguley, B. C.; Denny, W. A. *Anti-cancer Drug Design* **1993**, *8*, 429. (b) Palmer, B. D.; Lee, H. H.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1992**, *35*, 258.
7. Banik, B. K.; Venkatraman, M. S.; Mukhopadhyay, C.; Becker, F. F. *Tetrahedron Lett.* **1998**, *39*, 7243. Also see: (a) Banik, B. K.; Ghatak, A.; Venkatraman, M. S.; Becker, F. F. *Synth. Commun.* **2000**, *30*, 2701. (b) Banik, B. K.; Ghatak, A.; Mukhopadhyay, C.; Becker, F. F. *J. Chem. Res. (S)* **2000**, 108.
8. Banik, B. K.; Mukhopadhyay, C; Venkatraman, M. S.; Becker, F. F. *Tetrahedron Lett.* **1998**, *39*, 7247. Also see: (a) Banik, B. K.; Zegrocka, O.; Banik, I.; Hackfeld, L.; Becker, F. F. *Tetrahedron Lett.* **1999**, *40*, 6731. (b) Ghatak, A.; Becker, F. F.; Banik, B. K. *Tetrahedron Lett.* **2000**, *41*, 3793. (c) Basu, M. K.; Becker, F. F.; Banik, B. K. *Tetrahedron Lett.* **2000**, *41*, 5603. (d) Banik, B. K.; Suhendra, M.; Banik, I.; Becker, F. F. *Synth. Commun.* **2000**, in press.
9. Harvey, R. G.; Pataki, J.; Cortez, C.; DiRaddo, P.; Yang, C. *J. Org. Chem.* **1991**, *56*, 1210.
10. (a) Ranu, B. C.; Ghosh, K.; Jana, U. *J. Org. Chem.* **1996**, *61*, 9546. (b) Heaney, H. In *Comprehensive Organic Synthesis;* Trost, B. M., Ed.; Pergamon: Oxford, 1991; Vol. 2, p 753. (c) Olah, G. A. *Friedel-Crafts and Related Reactions;* Interscience: New York, 1964.
11. (a) Abu-Shqara, E.; Yang, C. X.; Harvey, R. G. *J. Org. Chem.* **1992**, *57*, 3312. (b) Minabe, M.; Cho, B. P.; Harvey, R. V. *J. Am. Chem. Soc.* **1989**, *111*, 3809. (c) Yoshida, M.; Hishida, K.; Minabe, M.; Suzuki, K. *J. Org. Chem.* **1980**, *45*, 1783. (d) Yoshida, M.; Minabe, M.; Suzuki, K. *J. Org. Chem.* **1979**, *44*, 3029. (e) Yoshida, M.; Nagayama, S.; Minabe, M.; Suzuki, K. *J. Org. Chem.* **1979**, *44*, 1915.
12. Jones, D. W.; Matthews, R. S.; Bartle, K. D. E. *Spectrochim Acta, Part A* **1972**, *28*, 2053.
13. Dewar, M. J. S.; Dennington, II, R. D. *J. Am. Chem. Soc.* **1989**, *111*, 3804. Chem 3-D Pro version 4.0 was used to obtain the starting geometries by doing MM2 calculations. DEWAR-PI was used to calculate the energies of the Wheland Intermediates in kcal/mol for substitution at each position. DEWAR-PI was available from the Quantum Chemistry Program Exchange (QCPE). Dewar, M. J. S.; Ruiz, J. M. *QCPE Bull.* **1988**, *8*, No. 1, 50.
14. Holzapfel, C. W.; Pettit, G. R. *J. Org. Chem.* **1985**, *50*, 2323.
15. Harvey, R. G.; Abu-Shqara, E.; Yang, C. X. *J. Org. Chem.* **1992**, *57*, 6313.
16. The reactions were carried out under a well-ventilated hood. Some of the compounds described here are potential carcinogens.