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Polycyclic Aromatic Compounds as Anticancer Agents: Structure–Activity Relationships of Chrysene and Pyrene Derivatives†

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Abstract—A large number of diamides and diamines were synthesized using 6-amino chrysene and 1-amino pyrene as starting materials. A structure–activity study with *cis*-platinum as internal control against animal and human tumor lines was carried out in vitro. This study indicated that the in vitro cytotoxicity toward these lines depends on the functionality present in the molecules. The diamino compounds were found to be more potent than the diamides, and these were equally active irrespective of the end heterocyclic group, whereas the activity of the diamides was strongly dependent on the terminal unit. In general, the diamides containing chrysene as the chromophore were more active than those with a pyrene ring. The size of the end heterocyclic ring, along with the nature of the spacer connecting the polycyclic ring to the heterocyclic ring, seemed to affect the biological activity in certain cell lines. Hemolysis experiments on a lead compound established that it had activities similar to those described for membrane-stabilizing agents. This agent also demonstrated the capacity to produce differentiation in leukemia cell lines.

Introduction

Synthesis of an enormous number of polycyclic aromatic hydrocarbons (PAH) by a variety of methods has been reported over many years.[1] The metabolic activation of these, their interaction with a spectrum of cellular organelles and macromolecules, and the resultant toxic and/or carcinogenic consequences have been described in detail.[2] Although the application of PAH derivatives as anti-cancer agents has also been explored,[3] far less is known regarding the basis for this important use. While this area of research has focused upon their interactions with the DNA-the system, and its replicative and repair constituents. It is increasingly recognized that other sites of activity may play a substantial role. This may also apply to the sites of action of a number of clinically utilized, as well as newly reported, chemotherapeutic agents. For example, Bair et al. recently reported the synthesis of a series of polyaromatic amino propane diol derivatives that demonstrate a close correlation between antitumor activity and the shape of the aromatic system. However, there was not as definitive a correlation as expected between the ability of these compounds to bind to DNA and their cytotoxic activity.[4] Similar studies raising questions concerning the exact targets for several newly synthesized agents have been published by Denny et al. and others.[5,6]

A striking example of this dichotomy in identifying 'the crucial' cellular target(s) is exemplified by the demonstration that adriamycin, when linked to an agarose support, is extremely active against the mouse leukemia L1210 without entrance into the cell suggesting some crucial interaction at the plasma membrane[7,8] — this, despite the vast number of reports indicating that the major focus of antitumor activity of this agent is at the level of DNA.

It has been shown that membrane-targeting depends upon a number of factors, including the charge and lipophilicity of the chemical agent, and that this interaction may result in selective alterations of membrane function.[9,10] A number of chemical agents that have a strong and possibly selective affinity for components of cell membranes and have been demonstrated to have anti-hemolytic[11] and hemolytic properties[12–16] at micromolar concentrations have been termed membrane-stabilizing agents (MSA). Numerous reports have suggested that their anti-hemolytic activity is the results of intercalation into and stabilization of the RBC membrane at low concentrations, possibly through a combination of

†Part 4 in this series. For Parts 1,2 and 3, see ref 19.

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lypophilic and charged interactions.[17] These compounds are exemplified by the phenothiazines, which protect the RBC against osmotic poration, while at higher, although still micromolar concentrations, their increasing intercalation decreases membrane order, and the stability of the RBC membrane and results in the leakage of hemoglobin. Phenothiazines also have important cell targets other than plasma membrane such as calmodulin and protein kinases. However, in view of their strong interactions with cell membranes, recent reports of their antitumor activities are pertinent to our current studies.[18]

With these findings as a template, we have synthesized a number of planar-molecules using highly lypophilic polycyclic aromatic amines as their nucleus for a systematic examination of modifications of structure, charge, and other characteristics that might result in primary, selective membrane interactions with cancer cells as a major factor in cell killing. In our preliminary publications, we demonstrated the synthesis and biological activity of chrysene derivatives against different cancer cells in vitro.[19a] We reported that modification of the terminal heterocyclic ring is important in determining the biological activity of these derivatives. However, we are aware that modifications of the aromatic rings and aliphatic linker could also influence cytotoxicity significantly. Based on these results, we undertook an exploratory study to evaluate the structure–activity of a series of new tetracyclic polyaromatic compounds. This paper reports in detail the synthesis of these compounds by a general and convergent route and the effects of these variations on several biologic modalities in addition to their antitumor cytotoxicity.

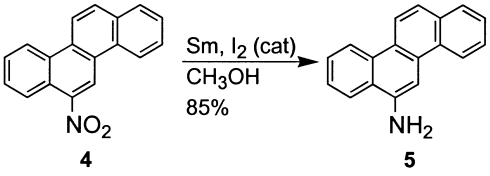
Chemistry

The acid **3** required for this present study was prepared by a general and highly efficient route. Condensation of the secondary amine **2** with cyclic anyhydride **1** in the presence of dichloromethane as the solvent under reflux temperature produced the acid **3** in more than 90% yield. We found it convenient to isolate the compound **3** directly after evaporation of the solvent and trituration with ether. Washing the organic layer with saturated sodium bicarbonate and acidification–extraction method gave poor yields of the product **3** presumably because of its partial solubility in water (Scheme 1).

Recently, we[20] developed a simple method for the reduction of the aromatic nitro compounds and imines to the amino compounds by samarium-induced iodine-catalyzed reaction.[21] For example, we prepared multigram quantities of 6-aminochrysene (**5**) by the reduction of commercially available 6-nitrochrysene (**4**) using this method (Scheme 2).

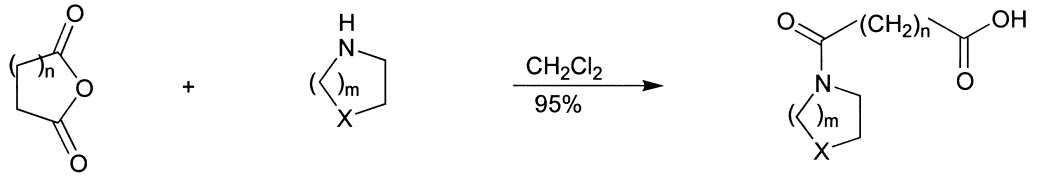
To link the acid to the aromatic system through nitrogen, our initial goal was to prepare several diamides, for example, **6** by coupling reaction. We reacted aniline and *p*-anisidine with the acid **3** in the presence of DCC and obtained the product amide in excellent yield. However, similar coupling of the acid **3** with 6-aminochrysene (**5**) in the presence of DCC[22] produced the diamide **6** in only 5% yield. This reflected a reactivity difference between monocyclic aromatic amines and polyaromatic amines, probably because of extended conjugation. However, the desired diamide **6** was prepared in excellent yield by a coupling reaction with the acid in the presence of isobutyl chloroformate-triethylamine.[23] We found that isobutylchloroformate-triethylamine is the best reagent system for this coupling reaction; several other condensing agents failed to produce the desired diamide **6** in reasonably good yield (Scheme 3).

By following an identical sequence, we prepared α,β-unsaturated diamide **9**. Thus, condensation of piperidine



4 **5**

Scheme 2.

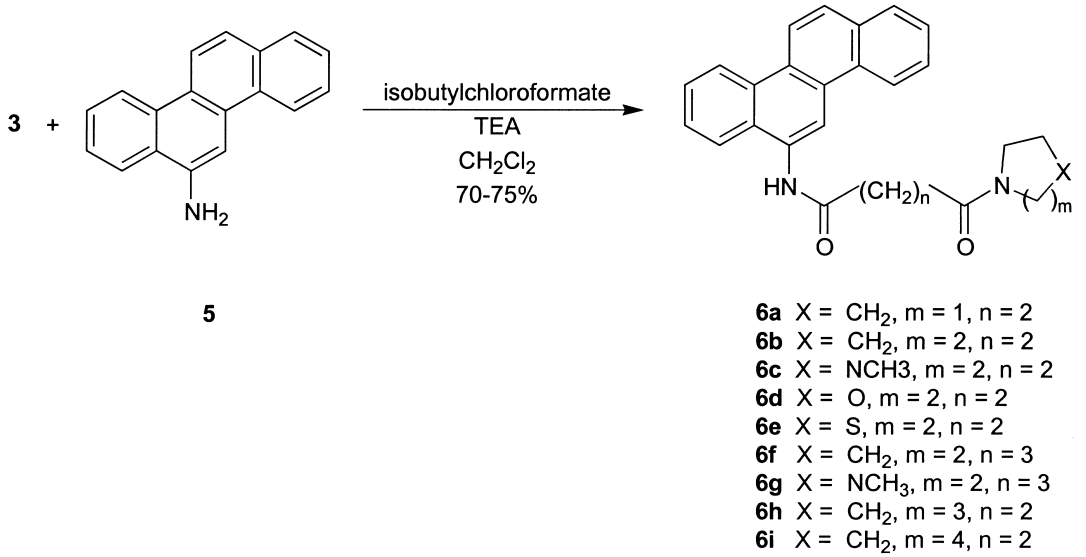


1a n = 1
1b n = 2

2a X = CH_2, m = 1
2b X = CH_2, m = 2
2c X = NCH_3, m = 2
2d X = O, m = 2
2e X = S, m = 2
2f X = CH_2, m = 3
2g X = NCH_3, m = 3

3a X = CH_2, m = 1, n = 2
3b X = CH_2, m = 2, n = 2
3c X = NCH_3, m = 2, n = 2
3d X = O, m = 2, n = 2
3e X = S, m = 2, n = 2
3f X = CH_2, m = 2, n = 3
3g X = NCH_3, m = 2, n = 3
3h X = CH_2, m = 3, n = 2
3i X = CH_2, m = 4, n = 2

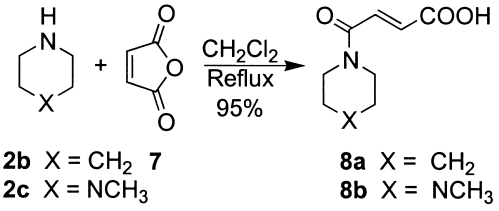
Scheme 1.



Scheme 3.

(**2b**) or *N*-methyl piperazine (**2c**) with maleic anhydride (**7**) produced the acid **8** in excellent yield. Condensation of the acid **8** with **5** under identical condition as described above afforded the *trans*-diamide **9** (Schemes 4 and 5).

Attention was then turned to preparing a 3-carbon spacer between the polycyclic aromatic and the heterocyclic rings. Towards this goal, we prepared the ester **11** by reacting **5** with ethylmalonyl chloride (**10**) in the presence of triethylamine. The ester **11** was hydrolyzed selectively by ethanolic sodium hydroxide and the resulting acid **12** was condensed with **2b** and **2c** in the presence of BOP[24] to produce **13**. Interestingly, the isobutyl chloroformate method failed to produce **13**. The structure of the product was deduced to be an isobutyl ester (Scheme 6).



Scheme 4.

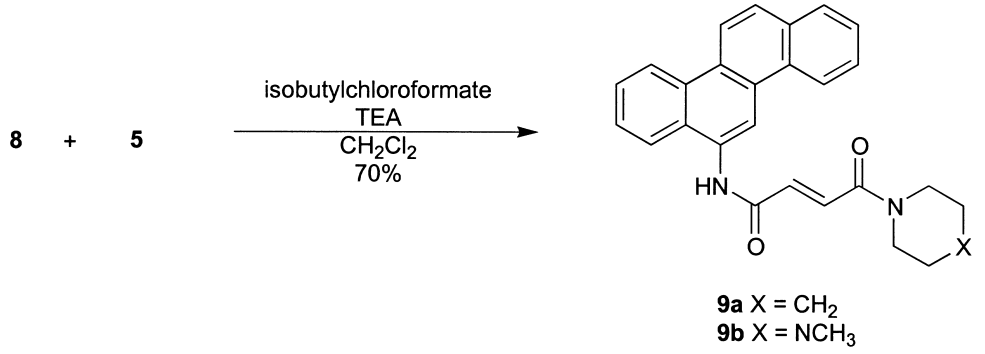
To establish the effects of the aromatic ring system on the biological activity, we prepared some pyrene derivatives using the chemistry described above. Thus, condensation of 1-amino pyrene (**15**) prepared by the reduction of 1-nitro pyrene (**14**) with **3a**, **3b**, **3c**, **3f** and **3g** using isobutylchloroformate-triethylamine method afforded the diamide **16** (Scheme 7).

The diamides **6b**, **6c** and **6d** were reduced by lithium aluminum hydride to get the diamines **17**, **18** and **19**, respectively (Scheme 8).

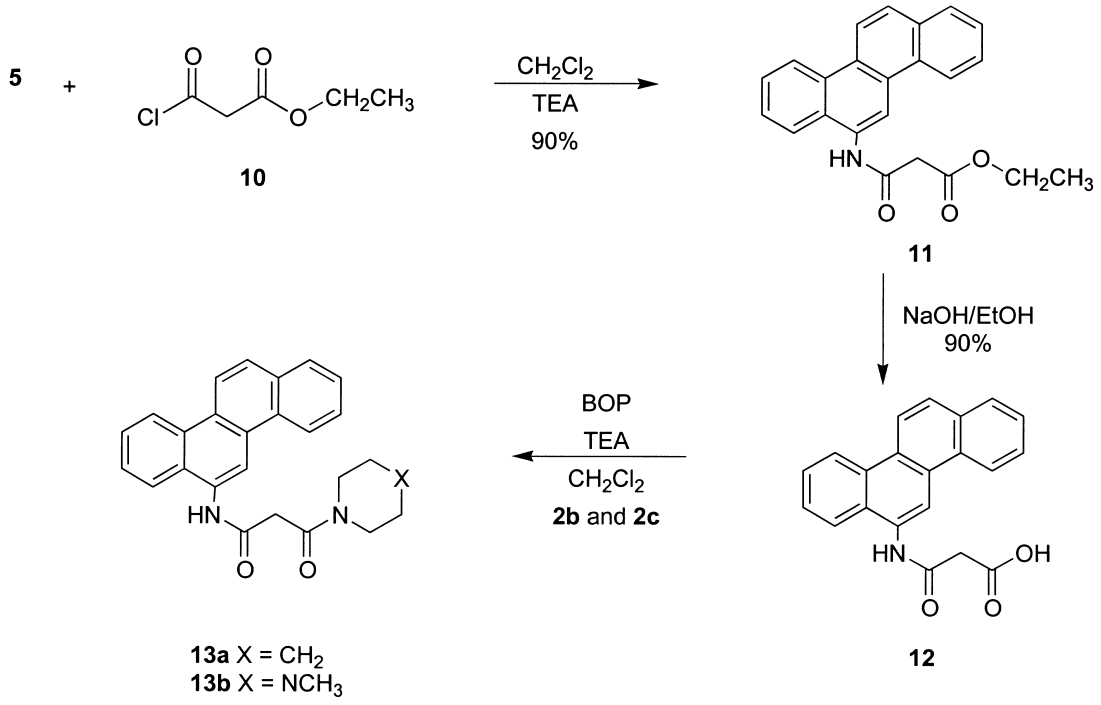
Cytotoxicity

Highly significant differences in cytotoxicity resulted from specific structural alterations in each of the three components of the molecules: the aliphatic linker, the aromatic ring, and the terminal heterocyclic ring (Tables 1–3).

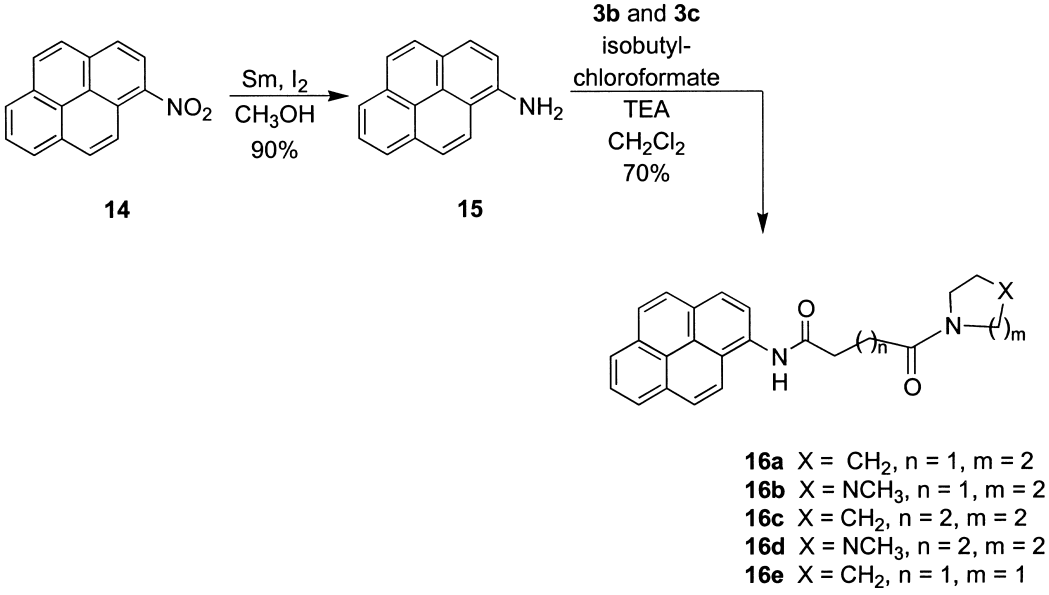
The most extreme alterations in activity of any described in this paper resulted from the reduction of the diamides in the linker of **6b** and **6c**. The resultant compounds, **17** and **18**, demonstrated IC_{50} cytotoxicity of 4 μM against the seven tumor lines tested. This considerable increase in activity was particularly striking for **6b**, the diamide,



Scheme 5.



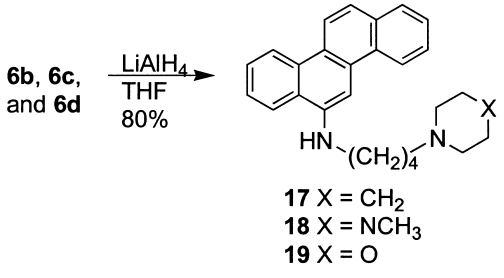
Scheme 6.



Scheme 7.

versus **17**, the diamine, wherein the former was deemed inactive (>20 μM) against all seven tumor lines. The reduction of the diamide groups in **6d**, a morpholine analogue of **6b** (Table 1) produced a remarkable increase in activity of the resultant compound **19**. While **6d** was inactive against all lines, **19** was active, or relatively active, against two, and the activity against the remaining five was dramatically increased.

The length of the spacer, however, did not appear to have a significant effect upon the activity of either compound.



Scheme 8.

Table 1.

Compound	No. runs	B_{16}	BRO	MCF-7	OVCAR3	$P_{388/0}$	PC_3	HT-29
cis-Platinum	2	7.33	5.66	15.99	—	—	1.66	15.99
6b	3	61.21	> 100	45.36	63.17	60.48	65.85	76.34
6c	3	14.58	33.64	40.0	18.11	8.70	27.29	16.70
6f	1	—	93.16	56.13	90.33	> 100	—	> 100
6g	1	—	25.74	31.66	12.75	14.80	—	17.99
9a	4	38.48	47.79	30.63	21.81	38.97	21.56	31.86
9b	3	17.25	30.49	14.89	9.92	17.96	—	12.52
13a	3	> 100	> 100	74.24	72.72	> 100	> 100	> 100
13b	1	—	24.08	14.59	29.68	19.70	—	23.60
17	3	3.66	4.18	4.71	4.18	3.92	3.92	4.45
18	1	4.03	5.03	4.03	4.53	4.78	3.77	4.53
19	1	5.20	14.58	15.36	12.5	4.16	13.28	14.84

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.

Thus, while the most consistent activity was demonstrated with the 4-carbon chain spacer, the substitution of a 3- or 5-carbon chain, **13b** or **6g**, produced similar cytotoxicity to that of **6c**. Little or no change was observed for the 3- or 5-carbon chain compounds **13a** and **6f** when compared with **6b**.

The insertion of a double bond in the spacer, as in **9b**, produced little change in the activity of **6c**, save for a trend over a number of runs towards an increase in activity against OVCAR. Insertion of an unsaturated bond in the spacer of **6b**, as in **9a**, had a more consistent effect. Overall, there was a trend toward increased activity against all tumor lines (Table 1), but none to active status.

When a pyrene aromatic ring moiety was substituted for chrysene, where the remainder of the structure was identical, such as in **16b** compared with **6c**, the former was inactive against all tumor lines, while the latter, the chrysene-based compound, was active against $P_{388/0}$, OVCAR-3, and HT-29. A similar contrast in activity was demonstrated for **16d**, the pyrene substituent for **6g**, where each had a 5-carbon linker.

Table 2.

Compound	No. runs	BRO	MCF-7	OVCAR3	$P_{388/0}$	HT-29
16a	2	57.81	67.70	45.57	56.51	48.95
16b	2	46.86	43.35	40.10	52.63	34.33
16c	2	> 100	> 100	30.90	51.25	37.68
16d	2	50.56	37.77	37.77	46.48	45.76
16e	2	> 100	—	35.13	59.18	45.13

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.

When pyrene was substituted for chrysene, as in **16a** and **16c** for **6b** and **6f**, no change in activity was induced, all of the compounds in this series remaining inactive (Table 2).

One of the most striking differences in activity between two closely related compounds was that between **6c** and **6b**. These compounds varied only in the terminal heterocyclic ring, the former an *N*-methylpiperazine and the latter a piperidine. **6c** was active (< 10 μM) against two of the animal-derived tumors, B_{16} and $P_{388/0}$, and against two human tumors, OVCAR-3 and HT-29. **6b** did not demonstrate activity against any of the seven tumor lines.

The effects of other alterations of the terminal heterocyclic ring were also tested (Table 3). Substitution of a morpholine (**6d**) or thiomorpholine (**6e**) for the piperadine ring of **6b** did not alter activity. Substitution of terminal heterocyclic rings of different size for the piperadine ring of **6b** had widely varied effects. An eight-membered ring, **6i**, produced no change from the inactive status of **6b** against all tested tumors. A five-membered ring, **6a**, had selective effect against OVCAR-3, where an increase on IC_{50} from 63.17 to 20.95 μM occurred. The substitution of a seven-membered ring, **6h**, produced a striking change in activity as well as a high degree of selectivity. While **6b** was inactive (approximately 50 μM or above) against all tumor lines, **6h** was active against B_{16} 19.57 μM, MFC-7, 21.22 μM, and OVCAR-3, 19.33

Table 3.

Compound	No. runs	B_{16}	BRO	MCF-7	OVCAR3	$P_{388/0}$	PC_3	HT-29
6d	2	> 100	> 100	> 100	68.44	> 100	—	48.54
6e	2	19.39	> 100	92.23	60.67	86.16	76.45	63.10
6a	1	—	> 100	88.38	20.95	> 100	—	> 100
6h	3	19.57	> 100	21.22	19.33	> 100	> 100	> 100
6i	2	> 100	> 100	38.99	88.50	> 100	> 100	> 100

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.

μM. The compound was totally inactive, >100 μM, against the remaining four tumor lines. This selective effectiveness of **6h** and of compounds such as **6c** that resulted in IC_{50}'s that varied from 8.70 μM and above against specific tumor lines is an important finding.

Hemolysis

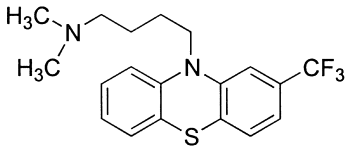
The phenothiazines trifluoperazine (Tf.per.) and triflupromazine (Tf.pro.) (Fig. 1) which have been used previously in numerous studies[14–16] for the investigation of the interaction of MSA with cell membranes, were used as controls and were prepared and tested as described for **6c**.

As a result of exposure to Tf.per. and Tf.pro., 50% hemolysis was achieved at concentrations of 1.6×10^{-4} and 5.0×10^{-4} M, respectively. These results agreed closely with prior reports and our previous studies. When tested under the same conditions **6c** produced 50% hemolysis at 1.4×10^{-4} M. As is apparent from these results, this concentration is even lower than that obtained with Tf.per., which has been described in a number of previous publications as one of the most active of the MSA[14–16] (Fig. 2).

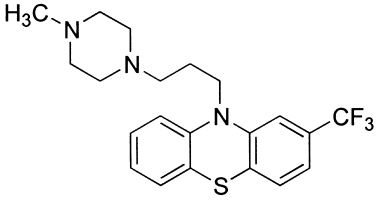
Cell Differentiation

We and others have utilized a number of tumor cell systems to demonstrate the induction of their differentiated cell forms. The erythroleukemias of mouse, DS-19, and human, K-562, respond to a variety of agents by the elaboration of hemoglobin.[25] The elaboration of hemoglobin in these cell lines has been taken as a sign of differentiation, and as such, has been used to demonstrate the effectiveness of diverse stimuli such as sodium butyrate and DMSO.

The application of appropriate differentiating agents to the promyelocytic leukemia line HL-60 results in their differentiation on either granulocytic cells or monocytes, depending upon the inducing agent.[26,27] Classically,



Triflupromazine



Trifluoperazine

Figure 1.

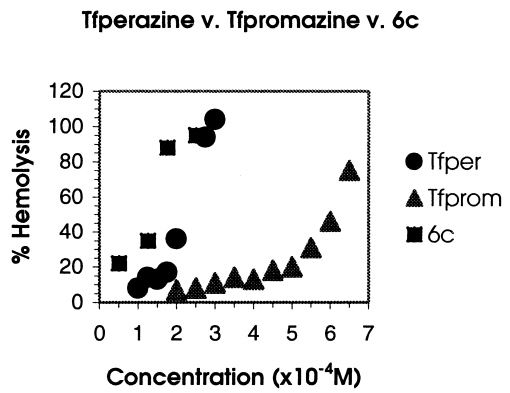


Figure 2. The percentage of hemolysis induced by T.f.prom. (▲), T.f.per. (●), and **6c** (■). The percentage of hemolysis was determined against a sample of red blood cells lysed with distilled water. Each of the curves was derived from at least three runs, and demonstrated significant consistency.

DMSO has been demonstrated to induce the granulocytic lineage, the degree of which is proportional to the concentration of exposure. We have utilized the ability of 'mature' granulocytes to elaborate superoxide, which converts the water-soluble NBT to insoluble blue formazan, as an identification of this lineage.

As one might predict from its cytotoxic activity against a number of tumor lines, and in particular those of hematologic derivation, **6c** demonstrated a sharply delineated threshold for toxicity (IC_{100}) which was found to be at 15 μg/mL and above against the cell lines to be tested for differentiation. **6b**, which is relatively inactive against the same tumor lines when applied in cytotoxicity assays, was less toxic when tested in suspension against K562 and HL-60. It required concentrations of 30 μg/mL and above of **6b** to induce cytotoxicity in these cell lines.

When **6c** was tested against the mouse erythroleukemia line DS-19, the development of hemoglobin by spectrophotometric assay was significant. Although there was variation from run to run, an increase of 55–56% in hemoglobin content was detected at levels for which viability remained reasonable. Against the human line K562, no hemoglobin induction was detected, while compound **6b** induced 49% increase in hemoglobin at 10.5 μg/mL, a concentration that did not reduce viability.

In a typical run with HL-60, **6c** at 10.6 μg/mL induced a positive tetrazolium reaction for superoxide production in 30% of the exposed cells, while an equivalent concentration of **6b** induced the reaction in 19% of the cells. However, at 25 μg/mL of **6b**, a concentration which reduced viability to 54% of the cells, 31% induction in the remaining cells was detected.

We then tested combinations of **6c** and DMSO as against the results of each singly. Exposing HL-60 cells to DMSO alone induced the maturation of cells as determined by the generation of peroxide molecules in the NBT test, ranging from 18.1% positive cells at 0.5% DMSO to 73.8% of the cells at 1.25% DMSO. Under these conditions, **6c** at a concentration of 6.5 μg/mL

produced a positive reaction in 26.9% of the cells without loss of viability. When 0.5% DMSO was added to each of the concentrations of **6c**, a striking increase in the total number of positive cells was detected for each combined regimen. For example, when 0.5% DMSO and 6.5 μg/mL were applied together, they induced 72.2% positive cells, a figure that far exceeded the 45% that would have been expected from simple addition of effect.

These results with DMSO plus **6c** suggest that there may be a more than additive effect that would imply that more than one pathway is involved.

Although the attempt to utilize tumor cell differentiation as an approach to cancer therapy continues to have strong support, we believe that this is another example wherein antitumor agents may affect multiple targets, depending on dose, exposure time, cell cycle, etc. The similarity in some respects of our polar planer molecules to the classic DMSO and others such as HMBA is of interest.

Toxicity

Compound **6c** administered at 30 mg/k, either by ip or iv routes, did not produce any severe toxicity or mortality when administered either at 1, 3, and 5 days, or 1, 5, and 9 days, respectively. The diamino derivative **17** was also tested against each mouse strain by several routes. Administration of 36 mg/k iv at 1, 2, and 3 days produced no mortality. At 72 mg/kg, an ip regimen of 1, 5, and 9 days produced 70% mortality, and while intravenously administered at 1, 3, 5, and 7, a 40% mortality.

Discussion

We have sought greatly to extend our initial studies of chrysene to determine if modifications of the aromatic and heterocyclic rings structure, as well as the aliphatic linker, would enhance their antitumor activity and offer additional insight into their mechanism(s) of action. It is evident from the literature that a number of relatively minor alterations in the structure of a pharmacological agent can drastically alter its cellular effect. Changes in the chemical and physical characteristics that such alterations may cause, such as charge, molecular configuration, and many others, have been variously offered as explanations for the differences in the specific cell target and efficacy. With many of these alterations, however, the suggested bases for these changes are speculative. Further, while in the field of cancer chemotherapy, the major focus for therapeutic effect has been upon alterations of the replicative and repair functions of the DNA-system, an increasing number of studies have suggested that interactions with other cellular constituents may play an important role in antitumor effectiveness. One of these areas of interest is the cell membrane.[7–10,28–30]

Almost no interaction between chemical molecules and cell components is as complex as those that take place with the plasma membrane. Here, exquisitely specific interference with surface receptors may take place, or as a result of intercalation into the membranes, alterations in ion flux may occur, lipid asymmetry may result and/or transmembrane functions may be disrupted. One of our lead compounds, **6c**, has been shown to interact with the membranes of red blood cells in a manner previously described for membrane-stabilizing agents. Indeed, it produced hemolysis at concentrations lower than one of the most active of the phenophiazines Tf.per., the group considered as model compounds of this membrane-interacting class. Based on numerous reports, it has been suggested that the phenothiazines intercalate into the membrane by hydrophobic interaction (possibly oriented via polar head groups of the fatty acids and the cationic moieties) until pore formation and hemoglobin leakage result. Although, with the data available, we cannot state with certainty that this is the site of the in vitro tumor cytotoxicity of **6c** or other compounds in the series, it does draw our attention to these interactions as potentially important to their effects.

It is of interest that a number of the agents we describe demonstrate both differentiating capacity and in vitro cytotoxicity. These cationic lipophilic structures would be expected to interact strongly with the membrane lipids and share with a number of the classic differentiating agents such as DMSO and HMBA, a polar planar structure.[27] While this circumstantially supports our concept that the focus of activity of this group of compounds may be 'nonspecific' or specific interaction with the membrane, it must be pointed out that DMSO and HMBA are not significantly cytotoxic in in vitro assays. Thus, the compounds described herein seem to represent the potential for a duality of effect. On the one hand, they induce differentiation as potently as classically used agents; on the other, their in vitro cytotoxicity, is similar to or even greater than that demonstrated by *cis*-platinum and certain other classic tumor agents believed to act primarily through cell damage and death.

The somewhat more than additive effect on differentiation when **6c** is used with DMSO suggests that there may be more than one mechanism involved in this response. Since in many instances the exact mechanism for invoking differentiation by a given agent remains unknown, this is an intriguing result. The duality of effect of the agents described herein may be another example where an antitumor agent affects multiple targets, depending on dose, exposure time, cell cycle, and other factors.

In terms of IC_{50}, the increase in the effectiveness of the lead compounds imparted by reduction of the diamide alkyl chain to diamine (as with **17**, **18** and **19**) is clearly the most potent chemical alteration of those reported herein. The resulting compounds were active against every tumor line tested, regardless of the prior IC_{50} of the diamide, some of which were inactive against all tumor lines.

The results indicate that the cytotoxicity of these compounds depends on the nature of the functional groups present in the molecules. Diamine compounds are more basic than the diamides and they can penetrate the cell

membrane much more efficiently because of their ability to form cationic intermediates. This hypothesis has been experimentally strengthened by our experiments in the diamide series, where the *N*-methyl piperazine containing compounds were consistently more active than those with other heterocyclic terminal ring structures. The end *N*-methyl group can also form cation intermediates and, theoretically, it is the most basic compound within the same family of this series. The lack of significantly differing cytotoxicities between diamines formed from compounds with *N*-methyl groups and others suggests that a maximum effect results from the diamide reduction.

Although the focus of these experiments was to determine the effect of a number of modifications of compounds synthesized on PAH moieties on in vitro tumor cytotoxicity, we feel it is necessary to comment on the maximal effectiveness achieved in this series. For example, while the activity of **17** was consistently in the 4.0 μM range, that of *cis*-platinum varied from more effective, 1.6 μM versus PC_3, to relatively inactive against HT-29 and MCF7, 15.99 μM. Other agents, such as adriamycin (ADR), are consistently more active than either compound. Although one goal of our ongoing research is to detect modifications of our lead compounds that will yield significant increases in their activities, it is accepted that absolute activity in vitro is often not proportional to clinical effectiveness. Thus, even though ADR is among the most potent antitumor agents in vitro and in vivo, and widely used clinically, it is well-recognized that its effectiveness against tumors in clinical applications is far from fully successful.

The lack of selectivity of the diamine compounds against specific tumor lines, when compared to **6c**, may suggest a toxic, rather than a cytotoxic, effect. However, one of these agents, **17**, has been shown to have acceptable levels of toxicity in vivo, and will soon be tested against engrafted tumors since ADR and other agents are also uniformly effective against all lines.

Although there may appear to be a contradiction in utilizing compounds that are closely related to known carcinogens/mutagens as antitumor agents, a number of factors strongly support this approach. First, there is a large body of evidence that modification of the structure of PAH can mitigate their carcinogenic/mutagenic potential, while taking advantage of interactions with specific cell components to emphasize their cytotoxic effects.[1,2,31,32] Thus, a broad spectrum of commonly used anti-cancer agents inclusive of carbazoles, anthracenes and others, as well as mutagenic mustards, are in current use clinically or in various phases of testing. Second, antitumor agents are rarely, if ever, administered over the long and continuous timeframe required for the carcinogenic effects that are characteristic of the PAH regimen. Thirdly, the seriousness of the clinical situation justifies the unquantified risk of using such agents, leaving them to be judged by the usual standards of clinical toxicity.

In conclusion, we are continuing to examine these and additional structural modifications to determine if our hypothesis, that an important component of their antitumor effect results from interactions with the cell membrane, is valid. We are also monitoring their potency as differentiating agents. In vivo testing against xenografted human tumors will also take place.

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. NMR spectra were recorded on Bruker 200 MHz and 300 MHz spectrometers. Chemical shifts are 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.

6-Aminochrysene (5). To a solution of 6-nitro chrysene (**4**, 500 mg, 1.96 mmol) in methanol (50 mL) was added Sm metal (601 mg, 4 mg atom) and iodine (102 mg, 0.4 mmol) under argon atmosphere. The mixture was refluxed for 8 h and then it was filtered through Celite. Methanol was removed under reduced pressure, and the residue was extracted with CH_2Cl_2 (100 mL), washed with $Na_2S_2O_3$ (10%, 10 mL), dried and then evaporated. The crude product on crystallization from ethanol afforded the pure 6-amino chrysene (**5**); yield: 90%, mp 210 °C, identical with the pure commercially available sample.

General procedure for the synthesis of the acids 3 and 8. To a solution of the anhydride **1** (0.2 mol) in dry CH_2Cl_2 was added the heterocyclic base **2** (0.2 mol) and the mixture was refluxed for 5 h. CH_2Cl_2 was evaporated and the crude product on crystallization from ether afforded the pure acid **3**. By following an identical sequence, and using maleic anhydride (**7**), acid **8** was prepared.

Butane-4′-carboxy-(1′-pyrrolidinyl)-carboxamide (3a). Melting point 96–98 °C. ^{1}H NMR ($CDCl_3$) δ 1.84–1.92 (quintet, 2H), 1.96–2.05 (quintet, 2H), 2.62–2.66 (m, 2H), 2.69–2.74 (m, 2H), 3.43–3.52 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 24.71, 26.32, 29.80, 29.85, 30.05, 46.43, 47.15, 171.21, 176.28. MS 172 (M^+). IR cm^{-1} (neat) 1724, 1600, 1471, 1456, 1343, 1229. Anal. ($C_8H_{13}NO_3$) C, H, N.

Butane-4′-carboxy-(1′-piperidinyl)-carboxamide (3b). Melting point 71–72 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–1.72 (m, 6H), 2.65 (s, 4H), 3.40–3.45 (t, 2H), 3.52–3.57 (t, 2H); ^{13}C ($CDCl_3$) NMR δ 24.78, 25.87, 26.66, 28.56, 30.30, 43.48, 46.96, 170.64, 177.25. MS 186 (M^+). UV (CH_2Cl_2) λ_{max} 227.21 (667). IR cm^{-1} (neat) 1716, 1622, 1445, 1253, 1227. Anal. ($C_9H_{15}NO_3$) C, H, N.

Butane-4′-carboxy-[1-(4′-*N*-methylpiperazinyl)]-carboxamide (3c). Melting point 86–88 °C. ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 2.48–2.70 (m, 8H), 3.50–3.61 (t, 2H), 3.61–3.72 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 28.56, 30.36, 40.78, 44.39, 45.11, 54.12, 54.33, 170.84, 176.90. MS 201 (M^+). UV (CH_2Cl_2) λ_{max} 227.10 (∈ 1016). IR cm^{-1} (neat) 3474, 1716, 1640, 1461, 1441, 1290, 1230. Anal. ($C_9H_{16}N_2O_3$) C, H, N.

Butane-4′-carboxy-(1′-morphonyl)-carboxamide (3d). Melting point 62–64 °C. ^{1}H NMR ($CDCl_3$) δ 2.56–2.78 (m, 4H), 3.50–3.66 (t, 2H), 3.68–3.75 (m, 6H); ^{13}C NMR ($CDCl_3$) 27.71, 29.33, 42.05, 45.65, 66.29, 66.59, 170.52, 176.68. MS 188 (M^+). UV (CH_2Cl_2) λ_{max} 227.26 (∈ 725). IR cm^{-1} (neat) 1728, 1627, 1439, 1273, 1233. Anal. ($C_8H_{13}NO_4$) C, H, N.

Butane-4′-carboxy-(1′-thiomorphonyl)-carboxamide (3e). Melting point 104–106 °C. ^{1}H NMR ($CDCl_3$) δ 2.60–2.72 (m, 8H), 3.75–3.80 (t, 2H), 3.87–3.92 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 27.73, 28.10, 28.40, 29.69, 45.10, 48.61, 170.50, 177.79. MS 204 (M^+). UV (CH_2Cl_2) λ_{max} 229.09 (∈ 976); IR cm^{-1} (neat) 1727, 1603, 1471, 1456, 1420, 1365, 1287, 1252, 1222. Anal. ($C_8H_{13}NO_2S$) C, H, N.

Pentane-5′-carboxy-(1′-piperidinyl)-carboxamide (3f). Melting point 54–56 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–1.89 (m, 6H), 1.86–2.00 (m, 2H), 2.39–2.46 (t, 4H), 3.38–3.43 (t, 2H), 3.52–3.57 (t, 2H). MS 200 (M^+). IR cm^{-1} (neat) 3418, 1723, 1591, 1445, 1368, 1252, 1224. Anal. ($C_{10}H_{17}NO_3$) C, H, N.

Pentane-5′-carboxy-[1-(4′-*N*-methylpiperazinyl)]-carboxamide (3g). Melting point 88–89 °C. ^{1}H NMR ($CDCl_3$) δ 1.80–1.98 (m, 2H), 2.30–2.43 (m, 4H with singlet at δ 2.38), 2.54–2.63 (m, 4H), 3.56–3.60 (t, 2H), 3.65–3.77 (t, 2H). MS 215 (M^+). IR cm^{-1} (neat) 3419, 1712, 1631, 1436, 1288, 1251. Anal. ($C_{10}H_{18}N_2O_3$) C, H, N.

Butane-4′-carboxy-(1′-hexamethyleneimine)-carboxamide (3h). Melting point 54–55 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–1.66 (m, 4H), 1.67–1.83 (m, 4H), 2.65–2.80 (m, 4H), 3.40–3.50 (t, 2H), 3.50–3.60 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 27.18, 27.33, 27.77, 28.49, 29.09, 30.12, 46.74, 48.35, 172.11, 177.09. MS 200 (M^+). UV (CH_2Cl_2) λ_{max} 227.05 (∈ 53). IR cm^{-1} (neat) 1713, 1599, 1436, 1376, 1246. Anal. ($C_{10}H_{17}NO_3$) C, H, N.

Butane-4′-carboxy-(1′-heptamethyleneimine)-carboxamide (3i). Oil. ^{1}H NMR ($CDCl_3$DE) δ 1.52–1.67 (m, 6H), 1.67–1.82 (m, 4H), 2.7 (s, 4H), 3.4–3.52 (t, 2H), 3.52–3.6 (t, 2H). MS 214 (M^+). IR cm^{-1} (neat) 1727, 1597, 1482, 1429, 1377, 1360, 1241, 1207. Anal. ($C_{11}H_{198}NO_3$) C, H, N.

But-2-ene-4′-carboxy-(1′-piperidinyl)-carboxamide (8a). Melting point 60–62 °C. ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 6H), 3.45–3.60 (t, 2H), 3.60–3.77 (t, 2H), 6.23–6.33 (d, 1H), 6.60–6.70 (d, 1H). MS 184 (M^+). UV (CH_2Cl_2) λ_{max} 233.19 (∈ 2158). IR cm^{-1} (neat) 1780, 1713, 1622, 1588, 1563, 1473, 1448, 1366, 1266, 1230. Anal. ($C_9H_{13}NO_3$) C, H, N.

But-2-ene-4′-carboxy-[1′-(4′-methylpiperazinyl)]-carboxamide (8b). Melting point 85–88 °C. ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 2.38–2.49 (t, 2H), 2.49–2.57 (t, 2H), 3.30–3.42 (t, 2H), 3.42–3.55 (t, 2H), 5.90–6.00 (d, 1H), 6.50–6.60 (d, 1H). MS 199 (M^+). UV (CH_2Cl_2) λ_{max} 228.14 (∈ 2408). IR cm^{-1} (neat) 3373, 1623, 1464, 1445, 1405, 1332, 1276, 1261. Anal. ($C_9H_{14}N_2O_3$) C, H, N.

General procedure for the synthesis of the diamides 6 and 9. Acid **3** (5 mmol) was taken in dry CH_2Cl_2 (100 mL), TEA (7 mmol) was added and the mixture was cooled at 0–5 °C. Isobutyl chloroformate (6 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise and the mixture was kept at 0–5 °C for 15 min. A solution of 6-amino chrysene (**5**, 5 mmol) in CH_2Cl_2 was added to it at the same temperature and the solution was stirred at room temperature for overnight. The mixture was washed with saturated $NaHCO_3$ (50 mL), brine, dried and solvent was evaporated. The crude material was chromatographed over silica gel using ethylacetate/hexanes (1/1) or methanol/ethylacetate (10/90) as the solvent.

***N*-(6′-Chrysenyl)-4-(1′-pyrrodinyl)-butane-1,4-dicarboxamide (6a).** Melting point 195–199 °C. ^{1}H NMR ($CDCl_3$) δ 1.85–2.01 (m, 4H), 2.81–2.87 (t, 2H), 2.97–3.03 (t, 2H), 3.43–3.50 (t, 2H), 3.54–3.60 (t, 2H), 7.58–7.73 (m, 4H), 7.91–7.98 (m, 2H), 8.25–8.30 (m, 1H), 8.62–8.67 (d, 1H), 8.76–8.83 (m, 2H), 9.47 (s, 1H), 9.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.31, 25.97, 31.07, 33.06, 46.08, 46.60, 114.11, 120.78, 121.75, 123.64, 125.74, 126.49, 128.28, 130.38, 131.10, 132.07, 132.26, 170.83, 172.07. MS 397 (M^+). UV (CH_2Cl_2) λ_{max} 271.0 nm (∈ 20108). IR cm^{-1} (neat) 3263, 1674, 1622, 1540, 1510, 1442, 1240, 1228. Anal. ($C_{26}H_{24}N_2O$): C, H, N.

***N*-(6′-chrysenyl)-4-(1′-piperidinyl)-butane-1,4-dicarboxamide (6b).** Melting point 184 °C. ^{1}H NMR ($CDCl_3$) δ 1.54 (broad s, 6H), 2.78–2.84 (t, 2H), 2.94–3.00 (t, 2H), 3.37–3.39 (t, 2H), 3.58–3.61 (t, 2H), 7.56–7.63 (m, 4H), 7.81–7.92 (m, 2H), 8.14–8.19 (m, 1H), 8.49–8.54 (d, 1H), 8.65–8.69 (m, 2H), 9.26 (s, 1H), 9.55 (s, 1H); ^{13}C NMR δ 24.33, 25.50, 16.20, 29.46, 43.09, 46.41, 114.52, 120.70, 121.76, 123.64, 126.31, 126.38, 126.44, 128.22, 130.34, 131.06, 131.97, 132.22, 170.38, 172.0. MS 411 (M^+). UV (CH_2Cl_2) λ_{max} 271.6 nm (∈ 143536). IR cm^{-1} (neat) 3260, 1623, 1539, 1511, 1471, 1440, 1224, 1203. Anal. ($C_{27}H_{26}N_2O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(4′-*N*-methylpiperazinyl)-butane-1,4-dicarboxamide (6c).** Melting point 216 °C. ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H), 2.44–2.52 and 2.57–2.65 (m, 4H), 2.80 (t, 2H), 2.90–2.96 (m, 2H), 3.57–3.62 (t, 2H), 3.78–3.80 (t, 2H), 7.58–7.72 (m, 4H), 7.91–7.96 (m, 2H), 8.15–8.20 (m, 1H), 8.62–8.66 (d, 1H), 8.73–8.93 (m, 2H), 9.18 (s, 1H), 9.38 (s, 1H); ^{13}C NMR δ 27.03, 30.46, 41.71, 42.17, 51.93, 52.18, 115.55, 120.64, 122.91, 123.10, 125.13, 125.88, 126.15, 126.37, 126.48, 126.78, 127.91, 128.91, 130.01, 131.26, 132.35, 169.71, 171.04. MS 426 (M^+). UV (CH_2Cl_2) λ_{max} 271.0nm (∈ 47143). IR cm^{-1} (neat) 3509, 3263, 1732, 1644, 1536, 1439, 1380, 1367, 1291, 1256, 1238, 1200. Anal. ($C_{27}H_{27}N_3O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-morphonyl)-butane-1,4-dicarboxamide (6d).** Melting point 234–235 °C. ^{1}H NMR ($CDCl_3$) δ 2.81–2.96 (t, 2H), 2.96–3.05 (t, 2H), 3.48–3.59

(t, 2H), 3.62–3.78 (m, 6H), 7.57–7.78 (m, 3H), 7.89–8.02 (m, 2H), 8.11–8.21 (m, 1H), 8.57–8.68 (d, 1H), 8.70–8.88 (m, 2H), 9.10 (s, 1H), 9.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.29, 32.93, 42.27, 45.76, 66.44, 66.77, 114.66, 120.79, 121.47, 123.68, 123.78, 125.95, 126.37, 126.54, 126.65, 128.30, 128.36, 130.36, 131.18, 131.70, 132.29, 170.91, 171.69. MS 413 (M^+). UV (CH_2Cl_2) λ_{max} 272.00 (46948). IR cm^{-1} (neat) 3261, 1647, 1630, 1539, 1512, 1438, 1362, 1300, 1272, 1235. Anal. ($C_{26}H_{24}N_2O_3$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-thiomorphonyl)-butane-1,4-dicarboxamide (6e).** Melting point 200–202 °C. ^{1}H NMR ($CDCl_3$) δ 2.55–2.70 (m, 4H), 2.80–2.93 (t, 2H), 2.93–3.07 (t, 2H), 3.73–3.88 (t, 2H), 7.55–7.75 (m, 2H), 7.90–8.00 (m, 2H), 8.10–8.20 (d, 1H), 8.60–8.68 (d, 1H), 8.70–8.82 (m, 2H), 9.08 (s, 1H), 9.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 27.81, 28.16, 30.01, 33.38, 45.15, 48.58, 115.16, 121.20, 121.87, 124.08, 124.20, 126.39, 126.96, 128.71, 130.77, 131.59, 132.09, 132.69, 171.08, 172.13. MS 429 (M^+). UV (CH_2Cl_2) λ_{max} 271.97 (∈ 39562). IR cm^{-1} (neat) 3267, 1628, 1539, 1509, 1464, 1441, 1366, 1288, 1240. Anal. ($C_{26}H_{24}N_2O_2S$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-piperidinyl)-pentane-1,4-dicarboxamide (6f).** Melting point 169–170 °C. ^{1}H NMR ($CDCl_3$) δ 1.62 (s, 6H), 2.17–2.23 (m, 2H), 2.59–2.66 (t, 2H), 3.46 (m, 2H), 3.63 (m, 2H), 7.59–7.73 (m, 4H), 7.92–7.97 (m, 2H), 8.23–8.28 (m, 1H), 8.63–8.68 (d, 1H), 8.75–8.85 (m, 2H), 8.97 (s, 1H), 9.39 (s 1H); ^{13}C NMR ($CDCl_3$) δ 21.77, 24.48, 25.63, 26.57, 32.04, 36.88, 42.79, 46.80, 115.03, 120.79, 121.60, 123.69, 123.74, 126.03, 126.45, 126.55, 126.63, 126.70, 128.28, 128.33, 130.36, 131.17, 131.74, 132.16, 170.96, 172.34. MS 425 (M^+). UV (CH_2Cl_2) λ_{max} 271.4 nm (86956). IR cm^{-1} (neat) 3252, 1651, 1635, 1538, 1510, 1479, 1466, 1441, 1417, 1276, 1260, 1226. Anal. ($C_{28}H_{28}N_2O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-*N*-methylpiperazinyl)-pentane-1,4-dicarboxamide (6g).** Melting point 176 °C. ^{1}H NMR ($CDCl_3$) δ 2.11–2.18 (m, 2H), 2.27 (s, 3H), 2.36–2.38 (m, 4H), 2.50–2.57 (t, 2H), 2.63–2.69 (t, 2H), 3.49 (m, 2H), 3.67 (m, 2H), 7.62–7.65 (m, 4H), 7.85–7.90 (m, 2H), 8.08–8.10 (m, 1H), 8.52–8.56, 8.64–8.72, and 8.79 (d, t, and s, 4H), 9.16 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.54, 31.98, 36.69, 41.52, 45.47, 45.90, 54.64, 55.07, 115.29, 120.76, 121.49, 123.61, 123.77, 126.10, 126.56, 126.67, 126.80, 128.20, 128.36, 130.33, 131.18, 131.56, 132.26, 171.15, 172.18. MS 438 (M^+). UV (CH_2Cl_2) λ_{max} 271.2 nm (32570). IR cm^{-1} (neat) 3255, 1651, 1623, 1540, 1440, 1291, 1253, 1237. Anal. ($C_{28}H_{29}N_3O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-hexamethyleneimine)-butane-1,4-dicarboxamide (6h).** Melting point 140 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–1.70 (m, 4H), 1.70–1.86 (m, 4H), 2.85–2.98 (t, 2H), 2.98–3.08 (t, 2H), 3.45–3.58 (t, 2H), 3.60–3.70 (t, 2H), 7.55–7.75 (m, 2H), 7.88–8.00 (m, 2H), 8.20–8.27 (m, 2H), 8.58–8.68 (d, 1H), 8.72–8.85 (m, 2H), 9.45 (s, 1H), 9.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.70, 27.01, 27.49, 28.68, 29.75, 33.23, 46.36, 47.95, 114.39, 120.75, 121.76, 123.58, 123.69, 125.78, 126.35, 126.40, 126.49, 128.25, 128.30, 130.34, 131.06, 132.01, 132.21, 171.95, 172.18. MS 438 (M^+). UV (CH_2Cl_2) λ_{max} 270.012 nm (∈ 83764). MS 425 (M^+). IR cm^{-1} (neat) 3265, 1674, 1619, 1540, 1510, 1478, 1439, 1240. Anal. ($C_{28}H_{28}N_2O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-heptamethyleneimine)-butane-1,4-dicarboxamide (6i).** Melting point 120–123 °C. ^{1}H NMR ($CDCl_3$) δ 1.48–1.82 (m, 10H), 2.92–3.0 (t, 2H), 3.0–3.06 (t, 2H), 3.44–3.47 (t, 2H), 3.52–3.59 (t, 2H), 7.59–7.71 (m, 3H), 7.91–7.96 (m, 2H), 8.24–8.25 (d, 1H), 8.63–8.65 (d, 1H), 8.76–8.81 (m, 2H), 9.46 (s, 1H), 9.61 (s, 1H). MS 439 (M^+). IR cm^{-1} (neat) 3265, 1725, 1674, 1620, 1539, 1511, 1477, 1441, 1359, 41332, 1286, 1236, 1206. Anal. ($C_{29}H_{30}N_2O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-piperidinyl)-but-2-ene-1,4-dicarboxamide (9a).** Melting point 162–163 °C. ^{1}H NMR ($CDCl_3$) δ 1.50–1.70 (m, 6H), 3.50–3.60 (t, 2H), 3.65–3.75 (t, 2H), 6.39–6.49 (d, 1H), 6.58–6.68 (d, 1), 7.55–7.79 (m, 3H), 7.90–7.99 (m, 2H), 8.35–8.41 (m, 1H), 8.61–8.68 (d, 1H), 8.72–8.85 (m, 2H), 9.60 (s, 1H), 11.05 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.15, 25.40, 26.25, 30.85, 42.74, 47.72, 114.39, 120.71, 123.53, 123.66, 125.97, 126.23, 126.37, 126.46, 126.52, 126.65, 128.16, 128.25, 129.11, 130.34, 131.03, 131.81, 132.19, 132.97, 162.83, 165.23. MS 409 (M^+). UV (CH_2Cl_2) λ_{max} 271.22 (43499). IR cm^{-1} (neat) 3243, 1683, 1667, 1603, 1543, 1510, 1475, 1442, 1410, 1393, 1366, 1275, 1259, 1241. Anal. ($C_{27}H_{24}N_2O_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(4′-*N*-methylpiperazinyl)-but-2-ene-1,4-dicarboxamide (9b).** Melting point 98–99 °C. ^{1}H NMR δ 2.29 (s, 3H), 2.40–2.51 (m, 4H), 3.61–3.66 (t, 2H), 3.76–3.81 (t, 2H), 6.43–6.64 (dd, 2H), 7.59–7.78 (m, 4H), 7.94–7.99 (m, 2H), 8.31–8.35 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 41.69, 45.84, 46.50, 54.34, 54.82, 114.50, 120.77, 121.89, 123.68, 126.11, 126.22, 126.48, 126.58, 126.64, 126.81, 128.22, 128.27, 128.34, 130.44, 131.13, 131.70, 132.27, 133.96, 163.67, 165.38. MS 424 (M^+). UV (CH_2Cl_2) λ_{max} 270.6 nm (∈ 39500). IR cm^{-1} (neat) 3240, 1667, 1614, 1543, 1510, 1472, 1441, 1290, 1259, 1241. Anal. ($C_{27}H_{25}N_3O_2$) C, H, N.

***N*-(1′-pyrenyl)-4-(1′-piperidinyl)-butane-1,4-dicarboxamide (16a).** Melting point 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 1.64 (m, 6H), 2.91 (m, 2H), 2.95 (m, 2H), 3.46 (m, 2H), 3.71 (m, 2H), 7.9-8.29 (m, 8H), 8.63 (d, 1H), 9.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.39, 25.60, 26.28, 29.80, 33.15, 43.23, 46.52, 120.83, 121.20, 122.70, 124.69, 124.80, 125.10, 125.14, 125.20, 125.98, 126.37, 127.37, 127.55, 128.43, 130.89, 131.38, 170.48, 171.97. MS 385 (M^+). UV (CH_2Cl_2) λ_{max} 244.2 nm (∈ 21134). IR cm^{-1} (neat) 3263, 1685, 1620, 1557, 1520, 1488, 1457, 1442, 1416, 1370, 1307, 1275, 1263, 1227. Anal. ($C_{25}H_{24}N_2O_2$) C, H, N.

***N*-(1′-pyrenyl)-4-(4′-*N*-methylpiperazinyl)-butane-1,4-dicarboxamide (16b).** Melting point 152–153 °C. ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.40–2.44 (t, 4H), 2.91 (m, 2H), 2.96–2.97 (t, 2H), 3.52–3.57 (t, 2H), 3.75–3.80 (t, 2H), 7.95–8.26 (m, 8H), 8.55–8.59 (d, 1H), 9.41 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.43, 32.75, 41.87, 45.20, 45.89, 54.51, 54.78, 120.77, 121.53, 122.96, 124.69, 125.05, 125.10, 125.96, 126.43, 127.27, 127.53, 128.53, 130.78, 131.09, 131.28. MS 400 (M^+). UV (CH_2Cl_2) λ_{max} 244.2

nm (60777). IR cm^{-1} (neat) 3261, 1643, 1627, 1600, 1557, 1520, 1488, 1460, 1437, 1416, 1290, 1274, 1266, 1261, 1229. Anal. ($C_{25}H_{25}N_3O_2$) C, H, N.

***N*-(1′-pyrenyl) 4-(1′-piperidinyl)-pentane-1,4-dicarboxamide (16c).** Melting point 138–139 °C. ^1H NMR ($CDCl_3$) δ 1.61 (s, 6H), 2.10–2.24 (m, 2H), 2.57–2.63 (t, 2H), 2.66–2.73 (t, 2H), 3.43–3.46 (t, 2H), 3.62–3.64 (t, 2H), 7.94–8.28 (m, 8H), 8.44–8.48 (d, 1H), 9.16 (s, 1H). MS 399 (M^+). UV (CH_2Cl_2) λ_{max} 244.2 nm (50026). IR cm^{-1} (neat) 3252, 1314, 1557, 1520, 1488, 1441, 1417, 1393, 1368, 1328, 1306, 1266, 1226. Anal. ($C_{26}H_{26}N_2O_2$) C, H, N.

***N*-(1′-pyrenyl)-4-(4′-*N*-methylpiperazinyl)-pentane-1,4-dicarboxamide (16d).** Melting point 171–172 °C. ^1H NMR ($CDCl_3$) δ 2.15–2.39 (m, 9H), 2.57–2.64 (t, 2H), 2.67–2.74 (t, 2H), 3.51–3.56 (t, 2H), 3.68–3.73 (t, 2H), 7.95–8.26 (m, 8H), 8.46–8.50 (d, 1H), 8.92 (s, 1H). MS 414 (M^+). UV (CH_2Cl_2) λ_{max} 244.4 nm (∈ 24214). IR cm^{-1} (neat) 3252, 1623, 1557, 1520, 1488, 1460, 1437, 1417, 1393, 1368, 1328, 1290, 1267, 1229. Anal. ($C_{26}H_{27}N_3O_2$) C, H, N.

***N*-(1′-pyrenyl)-4-(1′-pyrrolidinyl)-butane-1,4-dicarboxamide (16e).** Melting point 158–172 °C. ^1H NMR ($CDCl_3$) δ 1.99–2.14 (m, 4H), 2.93-99 (t, 2H), 3.09–3.15 (t, 2H), 3.56–3.62 (t, 2H), 3.69–3.75 (t, 2H), 8.08–8.30 (m, 7H), 8.41–8.46 (d, 1H), 8.71–8.75 (d, 1H), 9.93 (s, 1H). MS 371 (M^+). UV (CH_2Cl_2) λ_{max} 244.4 nm (16684). IR cm^{-1} (neat) 3260, 1668, 1620, 1557, 1520, 1488, 1449, 1416, 1373, 1342, 1327, 1307, 1269, 1227. Anal. ($C_{24}H_{22}N_2O_2$) C, H, N.

General procedure for the synthesis of the diamines. $LiAlH_4$ (10 mmol) powder was added to the diamide **6b** (5 mmol) in dry THF (60 mL) and the suspension was refluxed for 12 h. The mixture was cooled in ice and saturated Na_2SO_4 solution was added dropwise and then it was filtered and thoroughly washed with THF. After evaporation of the THF, the crude material was taken in CH_2Cl_2 (100 mL), washed with brine (10 mL), dried and evaporated. The pure product **17** was obtained after scratching the crude material with ether and hexanes. By following the above procedure, the reduction of **6c** and **6d** were achieved. The products **18** and **19** were obtained in good yield.

***N*-(6′-chrysenyl)-4-(1′-piperadinyl)-butane-1,4-diamine (17).** Melting point 101 °C. ^1H NMR ($CDCl_3$) δ 1.39–1.58 (m, 2H), 1.52–1.72 (m, 4H), 1.75–2.08 (m, 4H), 2.37–2.58 (t, 6H), 3.49–3.62 (t, 2H), 7.52–7.80 (m, 6H), 7.89–8.07 (m, 2H), 8.54–8.63 (d, 1H), 8.65–8.76 (d, 1H), 8.76–8.85 (d, 1H). MS 400 (M^+). UV (CH_2Cl_2) λ_{max} 281.98 (30678). MS 383 (M^+). IR cm^{-1} (neat) 3426, 1617, 1595, 1533, 1508, 1444, 1383, 1295, 1232. Anal. ($C_{27}H_{30}N_2$) C, H, N.

***N*-(6′-chrysenyl)-4-(4′-*N*-methylpiperazinyl)-butane-1,4-diamine (18).** Melting point 101–102 °C. ^1H NMR ($CDCl_3$) δ 1.70–2.03 (m, 8H), 2.32 (s, 3H), 2.39–2.68 (m, 6H), 3.49–3.59 (t, 2H), 7.52–7.80 (m, 6H), 7.88–8.03 (m, 2H), 8.53–8.63 (d, 1H), 8.68–8.75 (d, 1H), 8.75–8.84 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 24.87, 27.14, 44.15, 45.88, 53.00, 54.91, 58.07, 97.54, 120.32, 121.19, 121.56, 123.15, 123.23, 123.96, 124.41, 125.72, 125.76, 126.08, 126.59, 128.47, 129.66, 129.94, 131.34, 132.73, 142.17. MS 398 (M^+). UV (CH_2Cl_2) λ_{max} 279.11 (9900). IR cm^{-1} (neat) 3372, 1620, 1595, 1575, 1533, 1506, 1445, 1384, 1373, 1337, 1232. Anal. ($C_{27}H_{31}N_3$) C, H, N.

***N*-(6′-chrysenyl)-4-(1′-morphonyl)-butane-1,4-diamine (19).** Melting point 82–83 °C. ^1H NMR ($CDCl_3$) δ 1.74–2.08 (m, 8H), 2.43–2.58 (m, 4H), 3.50–3.60 (t, 2H), 3.70–3.80 (t, 2H), 7.55–7.80 (m, 6H), 7.89–8.03 (m, 2H), 8.55–8.62 (d, 1H), 8.65–8.72 (d, 1H), 8.75–8.82 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 24.46, 26.97, 44.04, 53.65, 66.88, 97.47, 120.18, 121.13, 121.50, 123.10, 123.14, 123.90, 124.33, 125.66, 126.02, 126.52, 128.42, 129.59, 129.85, 131.26, 132.67, 142.06. MS 385 (M^+). UV (CH_2Cl_2) λ_{max} 281.86 (14333). IR cm^{-1} (neat) 3409, 1619, 1594, 1533, 1506, 1445, 1385, 1275, 1260. Anal. ($C_{26}H_{28}N_2O$) C, H, N.

Synthesis of the ester 11. Ethylmalonyl chloride (**10**, 753 mg, 5 mmol) was added to a solution of 6-amino chrysene (**5**, 973 mg, 4 mmol) in dry CH_2Cl_2 (50 mL) and TEA (10 mmol) at 0–5 °C. The mixture was kept at rt for 4 h and then washed with dilute HCl (10 mL), brine (10 mL), dried and then evaporated. The crude product was used for the next reaction without any purification.

***N*-(6′-Chryseneyl)-propane-1′-carboxamide-3′-ethylcarboxylate (11).** Melting point 197–198 °C. ^1H NMR ($CDCl_3$ with drop of DMSO-d_6) δ 3.67 (s, 2H), 7.58–7.79 (m, 4H), 7.91–8.0 (m, 2H), 8.22–8.29 (m, 1H), 8.62–8.85 (m, 3H), 9.3 (s with shoulder at δ 9.32, 2H). MS 358 (M^+). IR cm^{-1} (neat) 3256, 1749, 1738, 1651, 1599, 1539, 1510, 1440, 1405, 1370, 1341, 1286, 1260, 1232, 1207. Anal. ($C_{23}H_{19}NO_3$) C, H, N.

Synthesis of the acid 12. The ester 11 (500 mg, 1.39 mmol) was hydrolyzed with ethanolic NaOH (112 mg, 2.79 mmol) at rt for 3 h. It was then extracted with CH_2Cl_2 to remove the traces of the unreacted ester. The aqueous part was acidified with cold HCl (1/1) and extracted with EtOAc (100 mL), washed with brine (10 mL), dried and evaporated. The acid 12 thus obtained was used directly without further characterizations for the next step.

***N*-(6′-Chryseneyl)-propane-3′-carboxy-1′-carboxamide (12).** Melting point 282–284 °C. ^1H NMR ($CDCl_3$) δ 7.55–7.78 (m, 4H), 7.9–8.0 (m, 2H), 8.1–8.19 (m, 1H), 8.59–8.68 (d, 1H), 8.7-8.85 (m, 2H), 9.48 (s, 1H), 10.19 (s, 1H). MS 330 (M^+). IR cm^{-1} (neat) 3238, 1725, 1650, 1598, 1547, 1514, 1481, 1463, 1439, 1410, 1336, 1276, 1260, 1238.

Synthesis of the diamide 13. To the acid **12** (100 mg, 0.30 mmol), dry DMF (8 mL), and methylpiperazine (50 μL, 0.45 mmol) at 0–5 °C was added successively HOBT (50 mg, 0.37 mmol), BOP (133 mg, 0.30 mmol), and *N*-methylmorpholine (50 μL, 0.45 mmol). After being stirred at rt for overnight, the mixture was diluted with H_2O, extracted with CH_2Cl_2, washed with H_2O, dried and evaporated. The pure product **13** was obtained after crystallization from EtOAc/hexane.

***N*-(6′-chrysenyl)-3-(1′-piperidinyl)-propane-1,3-dicarboxamide (13a).** Melting point 236–238 °C. ^{1}H NMR ($CDCl_3$) δ 1.72 (broad s, 6H), 3.58–3.67 (t, 2H), 3.70–3.81 (t with s at δ 3.69, 4H), 7.61–7.82 (m, 4H), 7.94–8.04 (m, 2H), 8.27–8.36 (m, 1H), 8.68–8.72 (d, 1H), 8.81–8.91 (m, 2H), 9.6 (s, 1H), 10.68 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.32, 25.55, 26.44, 29.70, 39.27, 43.32, 47.13, 113.56, 120.82, 121.47, 123.74, 123.78, 125.81, 126.07, 126.45, 126.52, 126.58, 126.69, 126.82, 128.33, 128.41, 130.42, 131.14, 131.77, 132.31, 165.17, 167.21. MS 397 (M^+). UV (CH_2Cl_2) λ_{max} 271.81 (∈ 38520). IR cm^{-1} (neat) 1694, 1610, 1554, 1524, 1479, 1466, 1445, 1388, 1375, 1353, 1305, 1283, 1250, 1219. Anal. ($C_{26}H_{24}N_2O_2$) C, H, N.

***N*-(6′-chrysenyl)-3-(4′-*N*-methylpiperazinyl)-propane-1,3-dicarboxamide (13b).** Melting point 170–172 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 2.45–2.58 (m, 6H), 3.65 (s, 2H), 3.75–3.85 (t, 2H), 7.57–7.82 (m, 4H), 7.91–8.02 (m, 2H), 8.2–8.3 (m, 1H), 8.62–8.68 (d, 1H), 8.74–8.89 (m, 2H), 9.54 (s, 1H), 10.85 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 39.37, 41.98, 45.82, 54.45, 54.86, 113.87, 120.78, 121.41, 123.69, 123.76, 125.91, 126.10, 126.47, 126.54, 126.72, 126.81, 128.31, 128.34, 130.37, 131.13, 131.56, 132.28, 164.87, 167.42. MS 412 (M^+). UV (CH_2Cl_2) λ_{max} 271.93 (43711). IR cm^{-1} (neat) 3253, 1644, 1548, 1513, 1440, 1387, 1335, 1292, 1240. Anal. ($C_{26}H_{25}N_3O_2$) C, H, N.

MTT colorimetric and in vitro cytotoxicity assays

The colorimetric assay was almost identical to that described by Chua et al.,[33] with the following modifications. In 3-day assays, 7000–8000 tumor cells/well were utilized and Trypan blue exclusion was applied to guarantee greater than 98% viability. Stock solutions of the test agents were prepared in DMSO/PEG300 (1/1), and the media that was used for serial dilutions contained 10% FBS. Care was taken so that the final concentration of DMSO did not exceed 0.1% at IC_{50}.

The NCI in vitro cytotoxicity assay described by these authors was also similar to that we utilized. The human-derived tumor lines we studied included BRO (melanoma), MCF-7 (breast), OVCAR-3 (ovary), PC_3 (prostate) and HT-29 (colon). These are representative of many of the human tumors that contribute to the overall mortality worldwide. The animal tumors B-16 (melanoma) and $P_{388/0}$ (leukemia) are standard animal tumors used in testing. The controls for the assay were achieved by including in every run one of our previously reported, consistently 'active' agents, as well as adriamycin. In the majority of runs, one of our inactive agents was used as a negative control, but no run was considered as reliable unless at least one 'new' agent in that run demonstrated positive and another negative activity.

The consistency of the in vitro assay was examined by comparing the cytotoxicity of a given agent over a period of 18 months. For example, the multiple separate tests of **6c** over that period against OVCAR-3 and HT-29 had a maximum variation of IC_{50} of 2.5 μmol. These values were also consistent with in vitro cytotoxicity results we had derived at least 1 year before the current 18-month experimental protocol. Although there was occasional variation in the result of a given agent against a single tumor line, in the vast majority of instances, the drug's status remained within its original classification of activity.

Hemolysis

One of the standard methods of identifying those chemical agents termed MSA, which have a relatively strong, specific, and stable affinity for interaction with cell membranes, is to determine if they can produce hemolysis of red blood cells (RBC) at micromolar concentrations. To determine whether our compounds fell into this category, we used the method of Perkins et al.[34] with minor modifications. Four mL of blood was drawn from an outbred, Sprague Dawley rat, and after two washings in 10 volumes of NaCl (0.9%), Tris buffer (1.5%, pH 7.40), the RBC were exposed to graded hypotonic saline solutions for 15 min and then spun at 1500 rpm for 5 min at 4 °C. The supernatant was read at 540 nm to determine the concentration of red cells required to give a consistent reading at 50% hemolysis. A similar determination using graded concentrations of DMSO was performed to make certain that the concentration of DMSO in which the test agent would be added, 0.5% DMSO, produced no significant hemolysis. The test agent was dissolved in DMSO, diluted to the appropriate concentration with the wash solution, and after exposure of the RBC for 15 min at 24 °C, they were spun as described above. The supernatants were read at 540 nm and compared with that of cells that had undergone 100% lysis with distilled water.

Cell differentiation

To determine if compounds of this general structure were capable of inducing cell differentiation in tumor lines, three cell lines were utilized, as were standardized 'marking techniques' that identify specialized products indicative of differentiation. The details of these procedures have been described in prior reports,[25–27] as have the measurements of these end products. In brief, mouse and a human erythroleukemia lines, DS-19 and K562, respectively, were utilized, as was a classic differentiation-test cell system, HL-60, derived from a human promyeloblastic leukemia. The conditions for cell growth, harvesting in log phase, and preparation have also been previously described.[25–27] Each test compound was dissolved in DMSO and diluted to its final concentration with the appropriate cell media. The final concentration of DMSO during test incubation was 0.05%, which produced no differentiation in control incubations. After 96 h of exposure, the cells were spun, washed twice with media, and exposed to the respective analytic system. For DS-19 and K562 cells, a spectrophotometric method was utilized after the cells were lysed and spun. The final reading was at 540 nm against standard hemoglobin curves. The expression of NTB-positivity was used as a marker for granulocyte differentiation.[25–27]

Mouse toxicity

The standard procedure of our Pharmacology and Analytic Center is to test antitumor agents in vivo

against xenografted tumors in nude, immunosuppressed mice and/or conventional mice by a variety of routes and regimens. Therefore, the agents utilized in this study were tested by appropriate routes and regimen in one or both of these. The nude mouse Hsd: Athymic Nude-Nu was obtained from Harlan Sprague Dawley at 6 weeks of age and used between 7 and 10 weeks of age. Conventional BDF_1 mice were purchased from Charles River Laboratories at 6 weeks and used between 7 and 8 weeks of age.

The agent to be tested was suspended at its maximum solubility in DMSO and then tested at the maximum concentration in 0.1 mL of the DMSO solution. The appropriate regulations governing the use of experimental animals were adhered to at all times and approved by the Institutional Animal Care and Use Committee. The animals were observed daily, and any signs of toxicity, morbidity, or local damage were recorded. They were also weighed daily as another measure of the toxicity of the agent.

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References

1. (a) Harvey, R. G. *Polycyclic Aromatic Hydrocarbons*; Wiley-VCH: 1997. (b) Rice, J. E.; Cai, Z.-W. *J. Med. Chem.* **1993**, *58*, 1415. (c) Harvey, R. G.; Pataki, J.; Cortez, C.; Raddo, P. D.; Yang, C. *J. Med. Chem.* **1991**, *56*, 1210. (d) Rice, J. E.; Czech, A.; Hussain, N.; La Voie, E. J. *J. Org. Chem.* **1988**, *53*, 1775. (e) Chung, Y.-S.; Kruk, H.; Barizo, O. M.; Katz, M.; Lee-Ruff, E. *J. Org. Chem.* **1987**, *52*, 1284. (f) Lee, H.; Harvey, R. G. *J. Org. Chem.* **1988**, *53*, 4253. (g) Harvey, R. G. *Polycyclic Hydrocarbons and Carcinogenesis;* American Chemical Society: Washington, DC, 1985. (h) Clar, E. *Polycyclic Hydrocarbons*; Academic: New York, 1964.
2. (a) Baird, W. M.; Ralston, S. L. In *Chemical Carcinogens and Anticarcinogens*; Bowden, G. T., Fischer, S. M., Eds; Pergamon: Oxford 1997; Vol. 12, p 171. (b) Grover, P. L.; Hall, M. In *Chemical carcinogenesis and mutagenesis*; Springer-Verlag: Berlin, 1990; Vol. 1, p 327. (c) Sami, S. M.; Dorr, R. T.; Alberts, D. S.; Remers, W. A. *J. Med. Chem.* **1993**, *36*, 765. (d) Palmer, B. D.; Rewcastle, G. W.; Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1988**, *31*, 707. (e) Di Raddo, P.; Chan, T. H. *J. Org. Chem.* **1982**, *47*, 1427.
3. (a) Polyaromatic compounds as anticancer agents: Venitt, S.; Crofton-Sleigh, C.; Agbandje, M.; Jenkins, T. C.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3748. (b) Agbandje, M.; Jenkins, T. C.; McKenna, R.; Reszka, A. P.; Neidle, S. *J. Med. Chem.* **1992**, *35*, 1418. (c) Perni, R. B.; Wentland, M. P.; Huang, J. I.; Powles, R. G.; Aldous, S.; Klinbeil, K. M.; Peverley, A. D.; Robinson, R. G.; Corbett, T. H.; Jones, J. L.; Mattes, K. C.; Rake, J. B.; Coughlin, S. A. *J. Med. Chem.* **1998**, *41*, 3645. (d) Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins, T. C.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3253.
4. (a) Bair, K. W.; Andrews, C. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1991**, *34*, 1983. (b) Bair, K. W.; Tuttle, R. l.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1990**, *33*, 2385.
5. Denny, W. A.; Rewcastle, G. W.; Gaguley, B. C. *J. Med. Chem.* **1990**, *33*, 814.
6. Denny, W. A. *Anti-Cancer Drug Des.* **1993**, *8*, 429.
7. Tritton, T. R.; Yee, G. *Science* **1982**, *217*, 248.
8. Tritton, T. R. *Pharmacol. Ther.* **1991**, *49*, 293.
9. Tocanne, J. F. *Biochem. Et Biophys. Acta* **1988**, *940*, 197.
10. Wright, S. E.; Hines, L. H. *Chem. Biol.–Interact.* **1990**, *75*, 31.
11. Born, G. V.; Housely, G. M. *Br. J. Pharm.* **1983**, *79*, 481.
12. Aki, H.; Yamamoto, M. *Biochem. Pharm.* **1990**, *39*, 396.
13. Horie, T.; Sugiyama, Y. *J. Pharm. Dyn.* **1981**, *4*, 116.
14. Housley, G. M.; Born, G. G. R. *Proc. R. Soc. Lond.* **1983**, *219*, 83.
15. Chen, Y. H.; Huestis, W. H. *Biochim. Biophys. Acta* **1997**, *1323*, 299.
16. Kanaho, Y.; Sato, T. *Mol. Pharm.* **1981**, *20*, 704.
17. Sweet, W. D. *Biochemistry* **1987**, *26*, 2828.
18. Motohashi, N.; Kurihara, T.; Satoh, K.; Sakagami, H.; Mucsi, I.; Pusztai, R.; Szabo, M.; Molnar, J. *Anticancer Res.* **1999**, *19*, 1837.
19. (a) Becker, F. F.; Banik, B. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2877. (b) Banik, B. K.; Becker, F. F. *Curr. Med. Chem.* **2001**, in press. (c) Becker, F. F.; Mukhopadhyay, C.; Hackfeld, L.; Banik, I.; Banik, B. K. *Bioorg. Med. Chem.* **2001**, *8*, 2693.
20. (a) Banik, B. K.; Mukhopadhyay, C.; Venkatraman, M. S.; Becker, F. F. *Tetrahedron Lett.* **1998**, *39*, 7247. (b) Banik, B. K.; Zegrocka, O.; Banik, I.; Hackfeld, L.; Becker, F. F. *Tetrahedron Lett.* **1999**, *40*, 6731. Also see. Basu, M.; Becker, F. F.; Banik, B. K. *Tetrahedron Lett.* **2000**, *41*, 5603.
21. Huang, Y.; Zhang, Y.; Wang, Y. *Tetrahedron Lett.* **1997**, *38*, 1065.
22. Lee, M.; Lown, J. W. *J. Org. Chem.* **1987**, *52*, 5717.
23. Holzapfel, C. W.; Pettit, G. R. *J. Org. Chem.* **1985**, *50*, 2323.
24. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *14*, 1219.
25. Huang, Y.; Wang X.-B.; Holzel, R.; Becker, F. F.; Gascoyne, P. R. C. *Phys. Med. Biol.* **1995**, *40*, 1789.
26. Brackman, D.; Lund-Johansen, F.; Aarskog, D. *Leuk. Res.* **1995**, *19*, 57.
27. Breitman, T. R.; He, R. *Cancer Res.* **1990**, *50*, 6268.
28. Berdel, W. E. *Br. J. Cancer* **1991**, *64*, 208.
29. Perez-Solar, R. *Cancer Res.* **1993**, *53*, 1583.
30. Werner, W.; Baumgart, J. *Biophys. Chem.* **1990**, *35*, 271.
31. Freudenthal, R., Jones. P. W., Eds.; *Carcinogenesis*; Raven: New York, 1976; Vol. 1.
32. Gelboin, H. V., (Ed.) *Polycyclic Hydrocarbons and Cancer*; Academic: New York, 1978; Vol. 1.
33. Chua, M. S.; Shi, D. F.; Wrigley, S.; Bradshaw, T. D.; Hutchinson, I.; Shaw, P. N.; Barrett, D. A.; Stanley, L. A.; Stevens, M. F. G. *J. Med. Chem.* **1999**, *42*, 381.
34. Perkins, W. R.; Dause, R. B.; Li, X.; Franklin, J. C.; Cabral-Lilly, D. J.; Zha, Y.; Dank, E. H.; Mayhew, E.; Janoff, A. S. *Biochem. Biophys, Acta* **1997**, *1327*, 61.